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Case report

Intraoperative squash Cytology of diffuse glioma not otherwise specified, of the Cerebellum

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Abstract. *Objective:* Diffuse glioma arises anywhere in the CNS, but most frequent in the cerebral hemispheres. The tumor tends to be seen in children and in younger adults aged 20-30. We report one such case in an older female patient presenting the intraoperative cytology of the tumor. *Case report:* A 48-year-old female was diagnosed by MRI with a tumor of cerebellum. Cytologic material was obtained during the resection of the tumor and diagnosed cytologically as glioma. *Conclusion:* This case is presented to focus the ability of the intraoperative cytology in diagnosis of the glioma, using immunocytology and confirmed by histo- immunohistology. (www.actabiomedica.it)

Key words: Glioma cytology, histology.

Introduction

The updated 2016 edition of the World Health Organization (WHO) Classification of Tumours of the Central Nervous System (CNS) uses molecular parameters and the histology to define the main tumor categories. Major reclassification was made with regard to diffuse gliomas, medulloblastomas and other embryonal tumors. In this aspect after the histological confirmation of astrocytoma, the second indicator for adult patients is the presence or absence of isocitrate dehydrogenase (IDH1 or IDH2) mutations and 1p/19q status. A tumor (in adults) with oligodendroglial morphology, showing an IDH mutation but no 1p/19q loss, will be designated astrocytoma, IDH mutated. For diffuse astrocytoma without IDH mutations, the term "IDH wild type" is used . If molecular testing for IDH status could not be completed or was inconclusive, the term "not otherwise specified" (NOS) is used. Recent studies have focused on other genes expression as well (1-4).

Diffuse gliomas tend to be seen in younger adults aged 20-30 and in children (5). They arise anywhere in the CNS, but most frequent in the cerebral hemispheres. Some gliomas involve a large part of the brain or the entire CNS in a diffuse fashion (gliomatosis cerebri). Most pontine and medullary gliomas are diffuse. Histologically, the tumor cells can be stellate, spindle-shaped with fiber like processes, or plump with a large eosinophilic mass (6).

The cytology of diffuse glioma is characterized by slight hypercellularity, mild nuclear enlargement, and absence of mitotic activity and necrosis (7).

Here we report an intraoperative cytologic diagnosis of a diffuse glioma confirmed by histology**.**

Case Report

A 48-year-old female patient hospitalized at University Hospital of Heraklion Crete suffering from cranial pain and unsteadiness. She was subsequently diagnosed by MRI with a tumor of cerebellum. Past, personal and family history was free.

The hematological and biochemical parameters were within normal limits.

Squash cytology

Intraoperative squash smears were prepared as follows: 1-2 mm³ 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 (8). 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 artifacts by frozen section analysis (9). Squash cytology smear method has improved diagnostic efficacy and is an adjunct, reliable simple and cheap tool for the neurosurgeon in the operating theatre (10).

Results

Cytology

The air dried smears were used for Giemsa stain and immunocytochemistry, while the alcohol (60%) fixed for routine Papanicolaou stain (Fig. 1). Isolated elongated, epithelial-like neoplastic cells with oval nuclei and scanty cytoplasm were demonstrated. Mitoses and necrosis were not found. Glioma, ependymoma, medulloblastoma and teratoid/rhaboid tumor, were considered in the differential diagnosis.

Immunocytochemistry

Rapid immunocytochemistry for GFAP and cytokeratin on air fried smears took 22 minutes. and showed that the tumor cells were of glial origin: GFAP cytoplasmic positive (Fig. 2)

Additional antibodies for conventional ICC, included, S-100, synaptophysin, smooth muscle actin (SMA), desmin, and EMA. IDH-1 immunostain was not performed

Gross total resection of the tumor was performed by the neurosurgeon.

Histology: In histological specimens of the tumor, fixed in 10% formalin, the H&E stain was performed (Fig. 3).

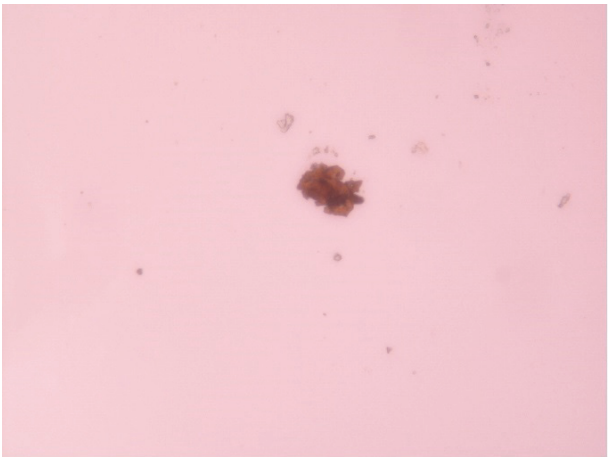

Figure 2. Grade-II Astrocytoma of the Cerebellum. Cytological squash smear. GFAP immunostainn x 400.

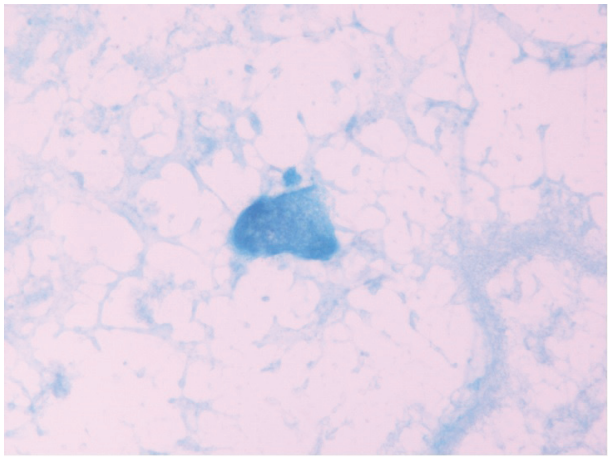

Figure 1. Grade-II Astrocytoma of the Cerebellum. Cytological squash smear. Papanicolaou stain x 400.

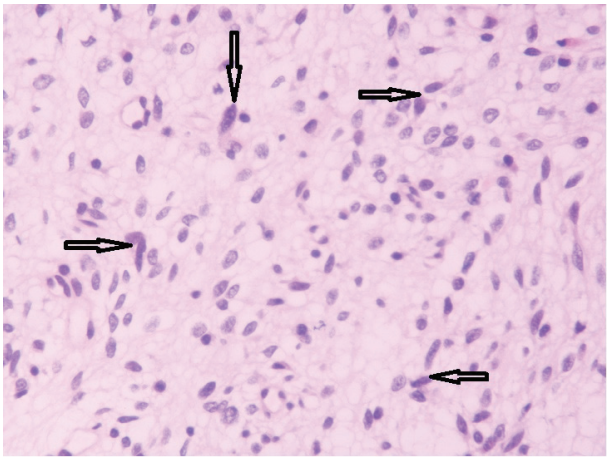

Figure 3. Grade-II Astrocytoma of the Cerebellum. Histopathology section. Hematoxylene-Eosin stain x 400.

Abundant material with cells of medium size with elongated or oval nuclei, extremely rare mitoses and neo-angiogenesis was observed..

Immunohistochemistry: The tumor cells expressed cytoplasmic GFAP (Fig. 4), S-100 and Vimentin but were negative for Synaptophysin, NF, EMA, CD34 and p53 markers. The proliferation index MIB-1 was found to be positive in 5% of tumor cells.

IDH(isocitrate dehydrogenase) and 1p/19q status were determined. Our case was a diffuse glioma not otherwise specified as IDH status was inconclusive. The patient received RT (54 Gy/30 fractions (fr)) followed by 6 courses of PCV (procarbazine, CCNU - lomustine, vincristine) chemotherapy. Five months after surgery she is disease free.

Discussion

Gliomas constitute a large and heterogenous group of tumors and notorious for wide differences in clinical incidence gross, microscopic features and biologic behavior.

Morphology cannot be accurate prognostic indicator where pathologists receive small biopsy samples but the gold standard remains histopathological examination of an abundantly sampled tissue (11).

The history of intraoperative cytology dates back to 1930, when Eisenhardt and Cushing introduced this technique by using supravital staining (12). The technique has recently gained importance because of advent of CT and MRI guided stereotactic biopsies. Cytology has been shown to be a great value in intraoperative consultation of CNS pathology (13) but the diagnosis of low-grade astrocytoma, especially grade-I fibrillary type, may be difficult to diagnose cytologically as well as histologically (14). The differentiation of normal, reactive and neoplastic astrocytes depends of identification of cytologic abnormalities. The astrocytes of reactive gliosis show cell processes and mild degree of cytologic abnormality that overlaps with slight degree of abnormality seen in low-grade astrocytoma (15). Low-grade astrocytomas show mild hypercellularity and slight cytologic atypia and pleomorphism. The biopsy from the central part of the tumor may be more representative but a biopsy taken from a peripheral area can be misdiagnosed cytologically due to sampling error (16).

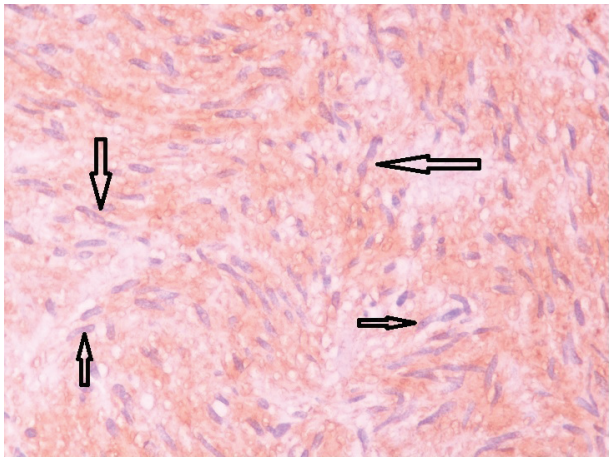

Figure 4. Grade-II Astrocytoma of the Cerebellum. Histopathology section. GFAP immunostain x 400.

Intraoperative cytology and frozen section are important in the diagnosis of neurosurgical samples. There are limitations in both procedures but understanding the errors and pitfalls may increase diagnostic accuracy. In a study by Chand P et.al. several technical errors were observed in the frozen sections, the principal being freezing process that changes the architecture, with sampling faults and misinterpretation coming next. In the crush smears, there was disagreement in diagnosis due to sampling and interpretative failure (17).

Intraoperative diagnosis of CNS lesions helps neurosurgical approach of these disorders. The perfect intraoperative process needs to be accurate, rapid, and must permit the maintenance of tissue for paraffin section analysis. Frozen sections provide structural details. Yet, the characteristic soft nature and high water content of the nervous tissue gives poor quality frozen sections. Consequently, different techniques such as squash smears and touch imprints are applied. Squash smears and touch imprints 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. Freezing artefacts can be prevented by most desirable temperature control and

prompt freezing of tissue. Touch imprints show better structural details than squash smear as crush artefacts are restrained (18-24).

Rapid immunocytochemistry is limited to some antibodies well documented in the literature such as keratins (25,26)

IDH-1 immunostain has values and limitations. It has been reported that it does not reliably identifies infiltrating tumor cells when admixed with preexisting or reactive glial cells and that it produces a non specific background staining (27).

In our case the use of intraoperative cytology not only helped the surgeon in rapid diagnosis of glioma, also it was ensured that minimum injury is caused to the normal brain structures surrounding the tumor.

In conclusion this report was undertaken to access utility of intraoperative smear cytology in diagnosing and grading glioma correlating and confirming by histopathological and immunohistological stains.

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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