ELECTRONIC CLINICAL CASE

Rapid cytologic diagnosis of choroidal malignant melanoma by vitreous smear

Le diagnostic cytologique rapide de mélanome malin choroidien par frottis vitréen

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Summary The eye is an uncommon subject of cytopathological examination. However, cytopathologic examination may be required for definitive diagnosis in some cases, as malignant tumors of the eye may sometimes be difficult to distinguish clinically from benign disorders. We report a case of malignant melanoma (MM) of the choroid, in which vitrectomy was performed for the initial clinical diagnosis of vitreous hemorrhage. As the dense vitreous hemorrhage was gradually cleared during the vitrectomy, a choroidal mass was discovered and the vitreous fluid was procured for rapid cytologic diagnosis. We used a modified Shorr’s stain that can be completed within several minutes. With this method, highly atypical, pleomorphic cancer cells, occasionally associated with melanin pigment granules, were demonstrated. These cytologic findings indicated a diagnosis of MM arising from the choroid. Histologic examination of the enucleated eye confirmed MM of epithelioid type. The advantage and indication of the rapid cytologic diagnosis is discussed.

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Résumé Il est rare de faire un examen cytopathologique de l’œil. Toutefois, cet examen peut s’avérer nécessaire pour le diagnostic précis : les tumeurs malignes peuvent parfois être difficiles à distinguer des lésions bénignes. Nous rapportons un cas de mélanome malin (MM) de la membrane choroidienne, dans lequel la vitrectomie a été réalisée devant le diagnostic clinique

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Introduction

Cytopathologic examinations are rarely performed for intraocular lesions, because invasive procedures are hampered due to the complicated, fine structure and important function of the eye. With the progress of imaging facilities, ophthalmologic diseases are now usually diagnosed clinically, whether malignant or benign. However, cytopathologic examination is sometimes necessary for a radiographically indeterminate lesion to make an unambiguous diagnosis [1].

We here report a case of choroidal malignant melanoma (MM), which was cytologically diagnosed on vitrectomy. The vitreous fluid was subjected for cytologic diagnosis using a rapid staining technique. With modified Shorr's stain, we were able to prepare and examine the specimens within several minutes, and could obtain a diagnosis of MM. The characteristics and advantage of this staining method are briefly discussed.

Case report

A 61-year-old man presented with a gradual decline of his visual acuity in his right eye, the cause of which had been diagnosed as vitreous haemorrhage by a local ophthalmologist. His visual acuity was 20/48 and 20/25 (right/left), respectively. His past and familial history was unremarkable, with no prior history of diabetes mellitus/hypertension. As an eye fundi examination failed to yield diagnostic information due to severe bleeding, an ocular ultrasonography was performed. It demonstrated ill-defined, dense opacity in the right eye, which was compatible with massive vitreous haemorrhage. No retinal detachment was identified.

In an attempt to resolve vitreous opacity and seek the source of bleeding, we performed three-port vitrectomy via pars plana using vitreous cutter under microscopic direct observation. Along with the clearing of the vitreous during the course of the operation, a pigmented choroidal mass was discovered. In an effort to diagnose this tumour immediately, vitreous fluid procured from vicinity of the tumour was subjected to cytopathologic examination. The vitreous fluid was briefly centrifuged (3,000 g × 3 min), cell palette was suspended with small volume of Saccomanno’s solution, smeared onto glass slides, fixed with absolute ethanol, and stained with Shorr’s stain [2] with some modification. Before the cytoplasmic stain with Shorr’s solution (Merck #1.09275.0500), we added extra steps of nuclear stain with Gill-Haematoxylin (30 seconds), enhancement in 37 °C water (several seconds), and differentiation (several dips in 0.5% hydrochloric acid in 70% ethanol) so that detailed nuclear/chromatin structures could be investigated. Smear specimens of vitreous fluid stained with this method revealed dyshesive, round to ovoid cells with marked cellular/nuclear pleomorphism associated with high nuclear/cytoplasmic ratio, and prominent nucleoli. Various amounts of melanin pigment granules were also identified. Bi-nucleated cells were observed, which comprised of about 5% of the total tumour cells (Fig. 1). These cytologic features suggested MM. After the vitrectomy, the diagnosis of choroidal MM was told to the patient. As no distant metastasis was detected, two treatment options were proposed: heavy particle external radiation or enucleation of the affected eye. After consultations of several ophthalmologic institutions to seek second opinions, the patient was finally consented to enucleation of his right eye at our hospital. The enucleation was performed 87 days after the initial diagnosis.

Cut section of the resected eye demonstrated a pigmented mass (14 × 8 mm) arising from lower part of the choroidal membrane, which was associated with intra-tumour haemorrhage and cystic change (Fig. 2). The tumor was larger than initially discovered on vitrectomy, perhaps because nearly three months had passed since the vitrectomy. Histologically, the tumour was epithelioid MM showing high atypia and pleomorphism (Fig. 3), and no extra-ocular extension was demonstrated. Immunohistostaining of HMG45 and S-100 was both positive (not shown). One year after the surgery, a systemic survey with positron-emission tomography demonstrated a positive nodule (φ ~ 10 mm) in his right lung, which was confirmed as metastasis by biopsy with video-assisted thoracic surgery of the lung (not shown). Bone metastases were subsequently discovered. The patient has been alive with disease for 16 months after the diagnosis, and systemic chemotherapy is considered.

Discussion

In the adult, choroidal MM is the most frequently observed primary intra-ocular tumour [3], which is associated with high risk of extra-ocular metastasis [4]. A prompt and accurate diagnosis is essential for appropriate management of this potentially life-threatening cancer. With the advance of imaging techniques in these days, intra-ocular tumour is usually diagnosed without invasive examinations.
[5]. Nevertheless, cytopathologic evidence of malignancy is occasionally needed when non-invasive imaging yielded only inconclusive results or the patient requested a pathologic evidence of malignancy before the treatment that potentially impairs visual acuity. As the eye is an organ of delicate, complex structure and also of important function, cytopathologic examination must be least invasive. This means that the amount of sample is usually small, so the samples must be processed to ensure the loss and artefact to be a minimum.

Initially employed trans-scleral biopsy was largely abandoned due to complications such as vitreous/sub-retinal bleeding and retinal detachment, and trans-vitreous fine needle aspiration (FNA) biopsy has been routinely utilised [6,7]. However, inadequate sampling is another problem [3]. To obtain diagnostic specimens, vitrectomy-assisted biopsies using vitreous cutter [1] and direct incision of the tumour using diamond knife [8] have been devised. These methods were developed to procure tissue samples large enough for the reliable histopathologic diagnosis while decreasing the risk of complications. During the vitrectomy, the most appropriate site of biopsy can be selected under direct observation. Post-biopsy bleeding, if occurred, can be managed by temporally increasing intra-ocular pressure [1] or endo-laser photocoagulation [8]. Vitrectomy-assisted biopsy can be changed to cytology in the operating room ad hoc if choroidal haemorrhage is expected [9]. As the cytopathologic diagnosis of MM shows excellent correlation with histologic examination [10], cytologic examination seems superior to histology in that it requires smaller amount of samples and less time/work. We decided to sample vitreous fluid around the tumour for rapid cytologic examination.

Our staining method was adapted from the single reagent stain of Shorr [2], to which we added a nuclear stain step with Gill-Haematoxylin. The addition of nuclear stain enabled detailed investigation of nuclei. Particularly, fine chromatin structures can be visualised. Nuclear stain enhancement was accelerated by immersing the slides in warm water. The time of nuclear stain/differentiation steps were optimised to maximise the demonstration of nuclear structure while keeping the staining time as short as possible. With this protocol, slides equivalent to conventional Papanicolaou (Pap) stain are reliably obtained. We have used this modified Shorr’s stain for more than 20 years in imprint cytology during the examination of frozen section as well as rapid on-site cytology of FNA/imprint cytology of core needle biopsy of breast lesions.

Liquid based cytology (LBC) has been widely used in clinical cytology practice, and its use in cytopathologic diagnosis of choroidal MM using vitreous fluid is reported [9]. LBC is superior to conventional smear method in that specimens
Figure 3. Histology of the resected tumour. The tumour was mixed with cystic change, haemorrhage, and solid tumour areas. Histologically, the tumour cells were composed of two different patterns. A. Smaller tumour cells with frequently associated with melanin granules. B. Larger tumour cells with ample cytoplasm. Melanin pigment was rarely observed within these areas. In both areas, the tumour was composed of loosely cohesive, pleomorphic, highly atypical cells with vesicular chromatin and eosinophilic nucleoli (Hematoxylin and eosin stain, × 200; bar = 100 μm).

with uniform distribution of cells and clear background are obtained. However, sample processing of LBC takes longer time for specimen preparation, and it harbours a risk of artefact. Preparation of samples from vitreous fluid with ThinPrep(R) required multiple steps to obtain adequate specimens [9]. In our method, only brief centrifugation (3,000 g × 3 min) and direct smearing of the cell pellet suspension over the glass slides were needed to prepare the specimens. This resulted in faster preparation and less artefact of the specimens. We were able to report the diagnosis MM to the referring ophthalmologist soon after the procurement of the vitreous fluid. When the cytologic findings of MM from various sites were compared, ThinPrep(R) slides tend to show coarser chromatin than those of conventional Pap stain, and loss of intra-nuclear inclusions was reported [11]. This means that some adaptation in the interpretation of cytologic findings is necessary for the diagnosis of LBC specimens. On the other hand, our modified Shorr’s stain yields almost equivalent specimens as conventional Pap slides. Thus, there was no need to adjust evaluation criteria of cytopathologic findings with our staining method. Our modified Shorr’s stain was useful in that it yields slides almost equivalent to Pap stain within a short time.

In summary, our staining protocol here reported enabled rapid yet reliable cytologic diagnosis. It is technically fairly simple and can be utilised in various clinical settings such as lymph node/thyroid/breast FNA cytology. Its use is advocated when immediate diagnosis is required for making clinical decision. Although ocular malignancy is uncommon, ophthalmologists should be aware of the available options for cytopathologic examination. And they should have close communication with cytopathologists to choose the most diagnostic method when encountered with a possibility of malignancy.

Disclosure of interest

The authors declare that they have no conflict of interest concerning this article.

References