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Reference


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Small incision iris tumour biopsy using a cavernous sampling forceps

Argyrios Chronopoulos, Ergin Kilic, Antonia M Joussen, Andreas Lipski

ABSTRACT

Background The aim of this retrospective report is to describe our experience with the Essen-23G biopsy forceps (Akgül forceps) for biopsies of pigmented iris tumours. Methods In this retrospective study of cases between October 2012 and September 2013, patients with iris tumours and clinical signs for malignancy underwent biopsy to secure the diagnosis. The Essen-23G-forceps was used to grasp and extract tissue through a clear corneal incision. Eventual entry and bimanual manipulation with a 23G mini-scissors was achieved through a second incision. Tissue samples were fixed in a sterile tube for further histopathological and immunohistochemical evaluation. Results Seven eyes of seven patients underwent biopsy using the forceps. The average thickness of the iris tumours was 1.07±0.79 mm. A second corneal incision for scissoring in a bimanual technique was necessary in 5 cases (71%). In 6 cases (85%), a precise histological and immunohistochemical diagnosis was achieved. Complications were limited to minute bleeding at the biopsy site and one case of relative pupil enlargement (anisocoria) without further refractive issues. Conclusions Iris tumour biopsies can be successfully approached using a cavernous 23G intraocular forceps with a low risk for procedure-related complications. The conical interior design allows for removal of whole tissue pieces with minimal manipulative artefacts. An optional bimanual access through a second corneal incision and use of a 23G scissors provides better efficacy.

INTRODUCTION

Pigmented iris lesions are common with a proportion of up to 68% of all iris tumours. Iris nevi represent the largest group with 51–62% of iridal pigmented lesions across all age groups.1 Contrastingly, iris melanoma is rare, representing only 2% of uveal tumours and 26% of solid iris tumours with a relative distribution of 8% in children, 16% in young adults, 20% in mid-adults and 19% in senior adults.1 2 The overall incidence of malignant melanoma of the iris has been reported to be 6.5 per 10 million per year.3 As iris melanomas can arise either de novo or from pre-existing nevi and account for life-threatening metastatic disease in about 10% of all cases, diagnostic criteria to secure the diagnosis in challenging cases are warranted.2 7–9

Besides excisional approaches, for example, sectoral iridectomy or iridocyclectomy,10–12 incisional biopsies and minimally invasive techniques such as fine-needle or cannula aspiration (FNA) using 23–27 gauge needles have been employed with different success and complication rates.13–17

In this context, we have employed a cavernous biopsy forceps to perform biopsies of iridal tumours. This method potentially enables retrieval of larger amounts of intact tissue with minimal trauma and complication risk. Here we report on our experience in pigmented iris lesions.

MATERIALS AND METHODS

All study procedures conformed to institutional guidelines and local ethics committee requirements. All patients stated their written informed consent. All consecutive iris tumour patients with clinical signs for malignancy presenting in our facility between October 2012 and September 2013 were offered a surgical biopsy. Only patients of one surgeon (AL) were included into analysis.

The biopsy forceps has been previously described in uveal tissue sampling.18 It is an intraocular forceps, specially designed for the purpose of precise and minimally invasive intraocular biopsies (23G/0.6 mm; D.O.R.C. Dutch Ophthalmic Research Center, 3214 VN Zuidland, The Netherlands). The inner surface of the branches has been specifically designed to enable grasping of tissue samples in a clear-cutting manner to avoid cellular damage to the specimen (figure 1). A single 1 mm clear corneal incision was applied under local anaesthesia in order to visualise any pigmented cells along the biopsy tract, and the anterior chamber was partially filled with viscoelastic.19 After careful inspection and determination of the biopsy site, the shaft of the forceps was introduced in the anterior chamber. Iris tumour tissue was grasped and eventually dissected into the cavity of the forceps. If necessary, a second paracentesis was applied for bimanual manipulation and controlled cutting of tissue with an intraocular scissors. The specimen was then carefully exerted through the anterior chamber with the forceps closed. Under microscopic control, the tissue specimen was rinsed with isotonic saline solution into a sterile 1.5 mL tube. After fixation with 4% formalin, the sample was submitted for further processing. Finally, the anterior chamber was rinsed with buffered saline solution in order to remove viscoelastic and pigmented remnants. Eventual bleeding at the biopsy site was tolerated if it terminated spontaneously. All procedures were conducted by the same surgeon. Postoperatively, all patients were re-examined within 24 h and in 3-month intervals following surgery.
GmbH, Hamburg, Germany. Secondary antibodies were S100 and Melan A were obtained from Dako Deutschland immunohistochemical evaluation. Antibodies against HMB 45, oedic acid –slides were stained with hematoxylin slides (DAKO Diagnostika GmbH, Hamburg, Germany). Four 69190 W alldorf, Germany) and transferred onto coated glass rotary microtome HM 360 (MICROM International GmbH, the specimen were prepared using an electronic motorised 1540 Chronopoulos A, after cooling of the paraf fibl xylol. After removal of the xylol supernatant, the tissue was with 96% alcohol, twice with 100% alcohol, and twice with xylol. After removal of the xylol supenrnatant carefully aspirated with a syringe. The tube was then filled with 70% alcohol and centrifuged again at the same rotation rate. The procedure was repeated with 80% alcohol, twice with 96% alcohol, twice with 100% alcohol, and twice with xylol. After removal of the xylol supernatant, the tissue was carefully placed into a metal specimen jar for paraffin embed- ding. After cooling of the paraffin wax, 2.5 μm-thick sections of the specimen were prepared using an electronic motorised rotary microtome HM 360 (MICROM International GmbH, 69190 Walldor, Germany) and transferred onto coated glass slides (DAKO Diagnostika GmbH, Hamburg, Germany). Four slides were stained with hematoxylin–eosin, and two with per- iodic acid–Schiff stain. Further unstained sections were kept for immunohistochemical evaluation. Antibodies against HMB 45, S100 and Melan A were obtained from Dako Deutschland GmbH, Hamburg, Germany. Secondary antibodies were included in the Dako REAL Detection System APAAP which was used for immunostaining. For staining of S100, monoclonal mouse antirabbit immunoglobulins from Dako were used as bridging antibodies. Negative controls were obtained by omis- sion of the primary monoclonal antibody.

RESULTS
The mean age of the two male and five female individuals (71.5%) was 61.1 ± 17 years. The mean interval from first examination to biopsy was 40.2 ± 44 months. Five patients were phacic and two (28.5%) were pseudophacic. Tumour thickness prior to intervention was 1.06 ± 0.79 mm (range 0.36 to 2.89 mm, table 1). All tumours were pigmented or partially pigmented and demonstrated one or more clinical signs of malignant transformation, such as increase of iris lesion size, increase of pigmentation, ectropion uveae, inferior localisation, intraocular pressure increment and feathery surface. A second corneal incision was necessary in 5 cases (71%). With the use of the bimanual technique, macroscopically visible biotic material of approximately 1×2 mm was obtained in all cases (n=7). The tissue was grasped by the forceps through the first incision and cut by a mini-scissors through a second incision if no tissue extraction was possible with the forceps alone. On the basis of the combined histological and histochemical approach, a precise diagnosis of malignant melanoma of the iris was made in 6 (8.5%) cases. The material was quantitatively insufficient in one case due to limited quality and yield of tissue in a dense and flat lesion. No definite exclusion of malignancy could be established in this case. Figures 2 and 3 depict examples of tissue workup from patients who underwent biopsy using the forceps.

COMPLICATIONS
No patient experienced a biopsy-related vision loss. Three patients developed temporary punctual bleedings but none of them ocular hypertension. The patient with the critical tissue gain developed a postoperative mild mydriasis and was adminis- tered a local pilocarpin therapy. No further complications, such as glare, refractive error, wound dehiscence, leakage, hyphaema or endophthalmitis were evaluable. A lens injury was avoidable in all the five patients with a phacic state.

DISCUSSION
Here we introduce a modified cavernous intraocular forceps for application in iris biopsies. Technically, the procedure can easily be performed in local anaesthesia. In order to avoid seeding along the biopsy tract a clear cornea incisional approach was chosen.19 The use of intracameral viscoelastics sufficiently helped to control anterior chamber depth and targeting of tissue as previously described.20

Table 1 Patient characteristics

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (years)</th>
<th>First presentation</th>
<th>Iris prominence in UBM (mm)</th>
<th>Marker reactivity</th>
<th>Histopathological diagnosis</th>
<th>Therapy</th>
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<td>1</td>
<td>47</td>
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<td>Uveal melanoma</td>
<td>SPBI</td>
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<td>Enucleation</td>
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<tr>
<td>7</td>
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<td>Apr 06</td>
<td>0.9</td>
<td>Melan A</td>
<td>Uveal melanoma</td>
<td>SPBI</td>
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</table>

SPBI, sectoral proton beam irradiation of iris; UBM, ultrasound biomicroscopy.

Figure 1 The Essen intraocular biopsy forceps. A conical design of the tip enables controlled extraction of whole tissue specimen from intraocular structures, for example, tumourous iris lesions.

SPECIMEN WORKUP
Specimen processing was performed as previously described.16 Briefly, the specimen was injected into a 50 mL tube supplemented with 20 mL 4% buffered formalin. After sufficient fixation, the fluid was centrifuged at 3000 rpm for 5 min and the supernatant carefully aspirated with a syringe. The tube was then filled with 70% alcohol and centrifuged again at the same rotation rate. The procedure was repeated with 80% alcohol, twice with 96% alcohol, twice with 100% alcohol, and twice with xylol. After removal of the xylol supernatant, the tissue was carefully placed into a metal specimen jar for paraffin embedding. After cooling of the paraffin wax, 2.5 μm-thick sections of the specimen were prepared using an electronic motorised rotary microtome HM 360 (MICROM International GmbH, 69190 Walldor, Germany) and transferred onto coated glass slides (DAKO Diagnostika GmbH, Hamburg, Germany). Four slides were stained with hematoxylin–eosin, and two with periodic acid–Schiff stain. Further unstained sections were kept for immunohistochemical evaluation. Antibodies against HMB 45, S100 and Melan A were obtained from Dako Deutschland GmbH, Hamburg, Germany. Secondary antibodies were used for immunostaining. For staining of S100, monoclonal mouse antirabbit immunoglobulins from Dako were used as bridging antibodies. Negative controls were obtained by omission of the primary monoclonal antibody.
The forceps enables a minimally invasive transcorneal sutureless procedure to recover sufficiently intact tissue for routine histopathologic examination and immunohistochemistry. However, persistent damage to pupillary constricting muscles may occur as it was the case in a patient with a plaque-type solid intrastromal tumour in which our method was not successful despite rigorous efforts to extract sufficient amounts of tissue. This could reflect an inherent limitation in dense iris tumours. In all other cases (n=6, 85%), a definite diagnosis of malignant melanoma of the iris could be established using our method.

Five patients underwent a modified iris sectoral proton beam irradiation (SPBI) within 6 weeks of the diagnosis. In one case with ring melanoma and optic atrophy due to secondary glaucoma, enucleation was performed to avoid further treatment-related and disease-related morbidity.

The short follow-up period in our study does not exclude tumourous cell dissemination prior or following our intervention. Whether early treatment is capable of preventing tumourous dissemination and metastatic disease remains unclear but appears likely when considering other melanocytic malignancies, for example, cutaneous, conjunctival and choroidal...
melanomas. However, no metastatic disease has been observed in our study. In the literature, the longest latency from treatment to metastatic disease in iris melanoma was reported to be 17 years. 

Previously, a variety of biopsy methods have been developed with different success rates. Since trans-scleral trephination of tumours was associated with high rates of extraocular tumour cell seeding,\textsuperscript{22, 23} it has been replaced by the FNA technique.\textsuperscript{5, 24} In its essence, FNA is a cytology procedure potentially leading to discrepancies in the interpretation of cytological results.\textsuperscript{5, 25} In an attempt to overcome this technical issue, vitrectomy cutting probes were used to ascertain larger amounts of tissue.\textsuperscript{16} Although technically successful, the procedure has been declared financially noneconomic. In this aftermath, further techniques have been developed with the objective to maximise tissue gain while minimising complications. Matthew’s et al\textsuperscript{15} employed a Rycroft transcorneal fine cannula aspiration technique in 12 patients with uncertain iris tumours which led to a histological diagnosis in all cases. Péer et al\textsuperscript{14} further reported good results with an approach of using a Kelly descemet’s membrane/trabeculectomy punch in 2 patients with iris tumours.\textsuperscript{14} By comparison, the cavity-confining, long-assembling blunt branches of the biopsy forces used in our series may enhance the applicability in small and/or flat iris lesions and thus help to avoid lens damage. Precise scissoring in a bimanual technique may suit controlled tissue extraction if necessary.

In summary, we present an alternative option to retrieve additional information in cases of uncertain iris tumours.\textsuperscript{26} However, the success of this method still depends on a thorough histopathological work-up and examination of the minute specimens by well-trained technicians and pathologists.

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Contributors All authors substantially contributed to the conception and data acquisition, and had access to all data. Authors AL and AC wrote the manuscript.

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