Review Article

Biopsy techniques for intraocular tumors

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Biopsy involves the surgical removal of a tissue specimen for histopathologic evaluation. Most intraocular tumors are reliably diagnosed based on the clinical evaluation or with noninvasive diagnostic techniques. However, accurately diagnosing a small percentage of tumors can be challenging. A tissue biopsy is thus needed to establish a definitive diagnosis and plan the requisite treatment. From fine-needle aspiration biopsy (FNAB) to surgical excision, all tissue collection techniques have been studied in the literature. Each technique has its indications and limitations. FNAB has been reported to provide for 88–95% reliable and safe ophthalmic tumor diagnosis and has gained popularity for prognostic purposes and providing eye conserving treatment surgeries. The technique and instrumentation for biopsy vary depending upon the tissue involved (retina, choroid, subretinal space, vitreous, and aqueous), suspected diagnosis, size, location, associated retinal detachment, and clarity of the media. The cytopathologist confers a very important role in diagnosis and their assistance plays a key role in managing and planning the treatment for malignancies.

Key words: Biopsy, cytology, eye, histopathology, malignancy, tumor

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The diagnosis of a disease could be etiological, tissue based, or of molecular basis. Biopsy is the histopathological/cytopathological evaluation of a tissue specimen after its surgical removal-in part or in toto. The role of procuring a biopsy is to provide a diagnosis and to estimate the prognosis. The first intraocular biopsy was performed by Hirschberg in 1868.[1] The techniques for intraocular biopsy have evolved over the years and serve to be imperative in the diagnosis and verification of malignant tumors before initiating any form of therapy.[2] In this article, we will discuss the indications, techniques, complications, and limitations of biopsy for intraocular tumors.

The main indication for intraocular tumor biopsy is when clinical examination and other investigations fail to establish an accurate diagnosis, e.g., tumor with atypical presentation, uveal metastasis with unknown primary tumor, or distinguishing a uveal melanoma from metastasis. It aids in determining the likely site of origin of ocular metastasis and in estimating the survival prognosis for the patient. The technique of obtaining a biopsy varies according to the site of the lesion involved. Biopsy techniques can be used for assessing both the anterior and the posterior segment tumors.

Biopsy Technique

The technique and instrumentation for intraocular biopsy can vary depending on the involved tissue, suspected diagnosis, location, size, presence of sub-retinal fluid, and clarity of the media.[3] The established methods of obtaining diagnostic material from tumors include the following:

a. Fine-needle aspiration biopsy (FNAB) [Fig. 1][2]
b. Surgical incisional biopsy
c. Surgical excisional biopsy.

Anterior Segment Tumor Biopsy Techniques

The anterior segment tumor accessibility through the cornea and limbus provides an easier approach to various techniques such as standard iridectomy, Finger iridectomy technique (FIT), and FNAB technique.[4] The various indications requiring a biopsy include rapidly enlarging iris mass, iris nevus, iris melanocytoma, primary iris melanoma, iris metastatic melanoma, iris pigment epithelial adenoma, and iris cyst.[2]

Aqueous tap

Aqueous tap can help in investigating neoplasms mimicking anterior uveitis or intraocular infections [Fig. 2], e.g., multiple myeloma, intraocular lymphoma. In cases of suspected retinoblastoma, it should be employed with extreme caution to prevent extra-ocular spread.[4] The ocular pathology laboratory is informed in advance, and the collected sample is immediately delivered to the laboratory. For adults, the procedure is done in the office with topical anesthesia (proparacaine 0.5%) and antibiotic cover (povidone-iodine 5%). The patient lies supine on the examination couch in the reclining position. The physician uses the illumination from indirect ophthalmoscope. This procedure can also be done on the slit-lamp microscope with

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Figure 1: Surgical tray layout with needles (left to right) 30-gauge, 27-gauge, 26-gauge of 1.5 inch length, tuberculin syringe with regular 26-gauge needle, 5cc syringe and 12cc syringe with attached tubing. The connector at distal end of the tubing can be attached to a needle

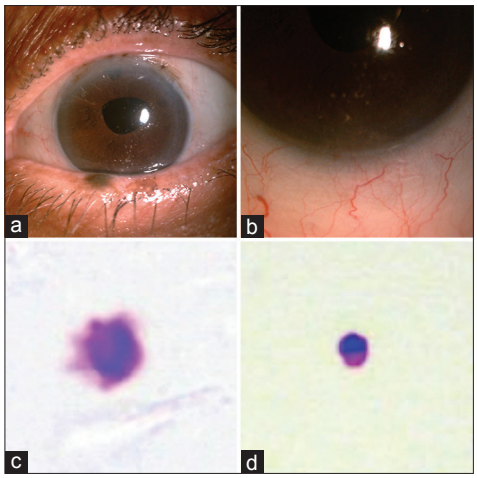

Figure 2: Anterior uveitis pseudo-masquerdae. (a) External photograph of right eye shows small and medium sized keratic precipitates in an 84-year-old male who underwent hemi-mandiblectomy for oral carcinoma, 3 years back and presented with bilateral anterior uveitis. (b) Higher magnification image of the eye with numerous keratic precipitates. Anterior chamber tap was done to rule out masquerade syndrome. Cytopathology revealed macrophages (c) and lymphocytes (d) but was negative for malignant cells

the patient in an upright position. After cleaning the periocular skin with povidone-iodine, a sterile speculum is used to keep the lids apart. A 1 ml syringe mounted with a 30-gauge needle is used to gain entry into the anterior chamber at the limbus and aspirate about 0.2–0.3 ml of aqueous and cellular debris. The needle is withdrawn while placing a sterile cotton-tipped applicator at the point of insertion. Gentle pressure is applied for 10–20 s at the limbal site of entry. A sterile eye-patch is applied. Complications include lenticular injury, corneal tract abscess, endophthalmitis.

Iridectomy and iridocyclectomy

Standard iridectomy and iridocyclectomy

It involves the local resection of the tumor along with the surrounding normal tissue margin [Fig. 3]. Indications for iridectomy include the following: Documented tumor growth, encroachment of tumor over pupillary margin or trabecular meshwork, malignant histology seen on FNAB specimen. The approach to surgical iridectomy may be limbal or sclerocorneal. The latter is preferred to avoid astigmatism. Para-limbal incision is made in the face of the tumor. A large incision is made so as to allow safe removal of tumor without brushing against adjacent tissues. Intracameral injection of miotic agent can help spare the pupil in certain situations. Viscoelastic is injected in the anterior chamber. Tumor is excised with 1–2 mm margins. The resultant iris defect can be repaired to reduce glare and diplopia. The main complications include glare, monocular diplopia, hyphema, cataract, infections, and intraocular pressure (IOP) fluctuations.[4]

The finger iridectomy technique

The cellular yield from FNAB is often limited to a few cells and carries the risk of tissue injury with the sharp needle tip and edges. On the other hand, surgical iridectomy requires a corneal wound and sutures, with subsequent healing. The FIT is a minimally invasive technique that involves creating a clear-corneal, self-sealing, juxta-limbal, 1 mm incision with a microvitreoretinal (MVR) blade, preferably on the same side of the tumor. Anterior chamber is stabilized with sodium hyaluronate 1%. A 25-gauge aspiration biopsy cannula (vitrectomy probe) is inserted in the anterior chamber to (at least partially) occlude the tumor with the aspiration port. The aspiration cutting is done at a suction of 300 m Hg and a cutting rate of 600 cuts/min. The specimen is collected by placing the cutter port in balanced salt solution (BSS) and the aspirate is flushed into a 5 ml syringe for cytopathological evaluation.[5] The potential complications are similar to iridectomy, but could also cause high IOP related to viscoelastic retention.

Fine-needle aspiration biopsy

FNAB is a novel technique for diagnosis and prognostication of ocular lesions. The technique depends upon the tissue involved, location, size, suspected diagnosis, and clarity of ocular media. Indications for FNAB include the following: Documented tumor growth or iris seeding with/without secondary glaucoma or multiple hyphema, to establish a tissue diagnosis before definitive treatment like enucleation or radiotherapy, presumed iris metastasis where systemic evaluation did not reveal a primary source.

It involves using a 26-gauge needle attached to a straight polyethylene connector tubing of 12–20 inches long which is attached to 10 ml syringe [Fig. 1]. The tubing prevents the transmission of small movements between the surgeon hands and syringe and thus provides stability. The length and volume of the tubing is important, as thin tubes provide higher resistance and large bore tubes provide inadequate suction. Tubing with elbows, clamps, filter, and joints are avoided because they obstruct, trap or dislodge cells. The length of tubing should be between 12 and 20 inches, as they provide an adequate surface area for cellular adhesion. Tubing with length <6 inches cause movement and interference with the needle tip in the eye.

The three considerations before planning FNAB are globe site entry, trans-aqueous course and tumor aspiration point. Except for children, FNAB can be performed under local

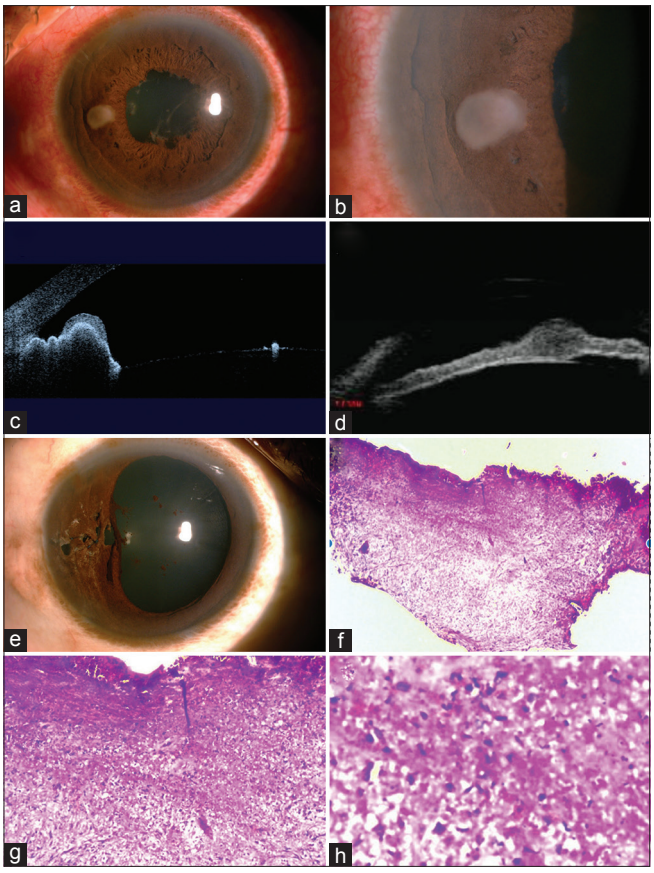

Figure 3: External photograph (a) of right eye shows circumcorneal congestion and greyish lesion in mid-peripheral iris of right eye. Slit-lamp photo (b) shows solid tumor of 2 mm × 1 mm size. Anterior segment optical coherence tomography (c) and ultrasound biomicroscopy (d) showed the tumor within iris stroma without extension to the posterior iris surface or angle. External photo (e) following excision biopsy of the tumor and primary repair of iris defect; pupil is spared. Histopathological examination (f-h) shows scattered lymphocytes and plasma cells without any granuloma formation (H and E, ×10, ×20, ×40)

anesthesia, using an operating microscope and using limbus as the site of entry and avoiding conjunctiva. The FNAB can also be performed under slit-lamp visualization by using cornea and limbus as the site for entry.

The site of entry is kept 90° from the meridian of tumor. After entering the anterior chamber, the needle is visualized with bevel side up and is passed through the aqueous parallel to the iris and into the tumor. The tumor aspiration point should be a relatively avascular site, at the thickest portion of the tumor, avoiding the lens. After securing entry into the tumor, gentle back and forth sliding motion of the needle along its trajectory within the mass is performed 3–4 times to loosen the cells for aspiration. Extreme care has to be taken while removing the needle so as to prevent anterior chamber flattening. The wound entry site is compressed with a gentle cotton tip applicator and intra-stromal hydration is done with BSS.

The aspirated cells are located predominantly in the needle tip and flushed into a syringe using BSS. The collected sample is then sent for cytopathological evaluation. Shields *et al*. conducted FNAB in 100 cases of anterior segment iris tumors and found 99% sampling yield for cytological evaluation with this technique.[6] The major complications include intraocular hemorrhage with secondary glaucoma, extra-ocular seeding, persistent hyphema, lens damage, prolonged hyoptony, and endophthalmitis.[7,8]

Posterior Segment Biopsy Techniques

For lesions of the posterior segment, the technical problems with biopsy techniques are much greater. The major indications for a biopsy for posterior segment lesion include differentiating malignant tumors from nonmalignant tumors, primary from secondary malignancy, investigating inflammatory and other diseases of the eye. The various techniques available for approaching the posterior segment lesions include:[9]

Surgical incisional biopsy

An incisional biopsy is a procedure wherein a small piece of tissue is taken to identify the composition of a lesion or abnormality. It has limited role in posterior segment tumor analysis and is reserved to a few choroidal/ciliary body tumors but plays a major role for anterior segment tumors. The tumor is approached by the partial thickness lamellar scleral flap and a sample of the tumor tissue is removed from within the deep scleral bed. The main advantage with incisional biopsy, is that it provides a larger sample than FNAB.[2]

Surgical excisional biopsy

The two approaches described in the literature include the trans-scleral and the trans-vitreal approach.[9]

Trans-scleral excision biopsy

The trans-scleral procedure involves the local resection of the tumor by partial lamellar sclerouvectomy (PLSU). Patient selection requires careful assessment of ocular and systemic findings. Recommended indications for resection of tumors include: Location of tumor in the ciliary body and/or peripheral choroid with no evidence of retinal invasion or vitreous seeding, tumors that measure up to 16 mm in largest tumor diameter, extend up to 7 mm posterior to the equator, and up to 5 mm in thickness, since these thicker tumors would require a more damaging dose of irradiation.[10]

The procedure involves achieving hypotensive anesthesia. The tumor is approached by partial thickness lamellar scleral resection and the tumor is removed along with deep scleral lamella along with the surrounding healthy choroid. The surgical technique involves outlining a hinged scleral/corneoscleral flap [Fig. 4a] that is dissected approximately 4 mm around the tumor [Fig. 4b and c]. An incision is made through the inner scleral fibers around the tumor to expose the uveal tract [Fig. 4d] and the tumor is removed along with the inner scleral fibers. The hinged scleral flap is resutured to its normal anatomic position [Fig. 4e]. In the recent years, the technique has been modified by using pars plana vitrectomy and silicone oil in case of breach in retinal integrity. Vitrectomy aids in volume reduction of the eyeball and thus provides easy access to posteriorly located tumors, even those abutting the optic disc and prevents retinal prolapse during the scleral closure. Fig. 5 depicts a case of CB-leiomyoma that was completely excised with PLSU using the trans-scleral approach.[10-12] Ramasubramanian *et al*. analyzed their results with this procedure in pediatric subjects, noting globe salvage in 65% and visual outcomes of 20/40 or better in 64%.[12] The challenges to performing this procedure include large

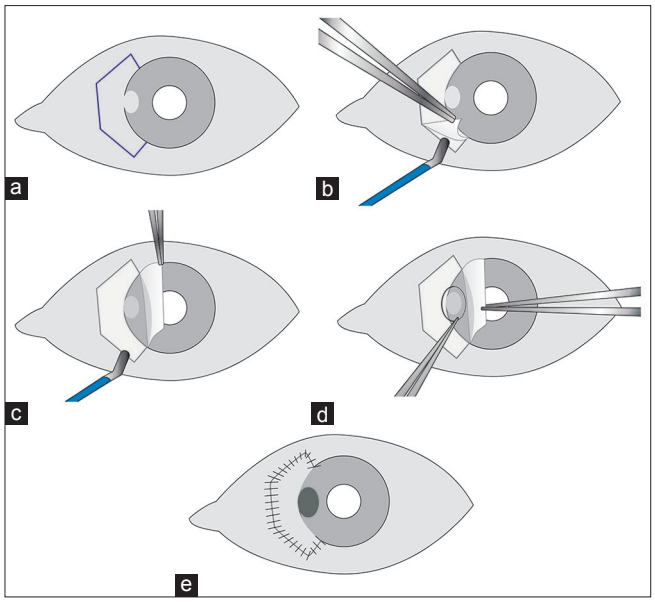

Figure 4: Schematic representation of the technique of partial lamellar sclerouvectomy for anterior uveal tumor. (a) front view shows the extent of the scleral flap over the tumor. (b) Initiation of lamellar dissection of the scleral flap. (c) The completely dissected and hinged corneoscleral flap. The shadow in the surgical bed depicts the basal dimensions of the tumor to be resected. (d) cutting through the inner scleral bed, underlying uveal tract and encompassing the uveal tumor, a complete resection is performed. (e) Outer scleral flap is sutured to its original position

tumor basal dimension, posterior location of tumor, tumor seeding into anterior chamber or vitreous, patients needing anticoagulation, and the surgeon's perception of the procedure as too difficult or too time consuming.

Complications encountered include transient hyphema or vitreous hemorrhage, retinal detachment, and expulsive hemorrhage. Vitreous surgery with endo tamponade is often employed for globe reconstruction. Despite being a difficult procedure to perform, PLSU remains the treatment of choice for several benign and malignant intraocular tumors. Enlarging benign tumors such as melanocytoma, retinal pigment epithelium adenoma, and ciliary body leiomyoma can destroy the globe and are not particularly radiosensitive or chemosensitive. Anteriorly located, well-defined malignant tumors may be best managed with resection, obviating the consequences of radiotherapy, e.g., iris or iridociliary melanoma, adenocarcinoma of the pigmented and nonpigmented epithelium, and selected metastatic foci such as carcinoid tumor.[11]

Trans-vitreal biopsy

The trans-vitreal approach is performed by standard three-port pars plana vitrectomy using micro-incision vitrectomy system and valved cannulae. After core vitrectomy, posterior vitreous separation is induced and a complete vitrectomy is performed. This helps avoid vitreoretinal incarceration with the vitreous cutter during the biopsy. An appropriate site over the tumor is selected for its avascularity and height. The IOP is elevated and a retinotomy incision is made into the tumor with MVR blade to allow the transretinal entrance of the vitreous cutter. The vitreous cutter is impaled into the tumor at the chosen site and cutting is activated at a low-cutting rate of about 100 cuts/min taking 2–4 bites from the tumor. The tissue material is aspirated from the cutter into a 5 ml syringe. The cutter is carefully removed from the tumor and gently withdrawn out. The sample is promptly sent for cytopathologic examination. The IOP is slowly decreased to the normal level, thus avoiding bleeding from the biopsy site. Fluid/gas exchange is performed; the choice of endotamponade depending upon the location of the tumor and presence of sub-retinal fluid. Retinopexy may not be required in all cases. A watertight closure of scleral incisions can be achieved with 7-0 vicryl sutures. The complications associated with the procedure are vitreous hemorrhage, sub-retinal hemorrhage, and an inconclusive biopsy sample.

Fine-needle aspiration biopsy

FNAB is an established and accepted technique for investigating and diagnosing lesions affecting the posterior segment. In 1979, Jakobiec published a major report on the use of FNAB for diagnosing intraocular tumors.[7] FNAB offers a 88–95% safety and reliability profile for diagnosing ophthalmic lesions.[7] The technique and instrumentation for FNAB for posterior segment lesions varies depending upon the structure involved (choroid, retina, subretinal, vitreous), size and location of tumor, and media clarity. The preequatorial and ciliary body lesions are approached trans-sclerally and postequatorial lesions are approached by trans-vitreal approach.[7]

Trans-scleral approach

The trans-scleral approach consists of making a 3-mm square scleral flap of 80% depth. A short 25–30-gauge needle is attached to a tubing. It is inserted through the scleral bed tangentially to obtain a sample. The scleral flaps are sutured. Young *et al*. conducted FNAB via trans-scleral approach for macular choroidal melanoma and concluded it to be a feasible technique for confirming the cytogenetic prognosis.[13] The complications reported with the trans-scleral technique are retinal detachment, submacular, and vitreous hemorrhage. Methods to reduce potential systemic dissemination of tumor cells from the needle puncture site include application of cryotherapy or cotton-tipped applicator soaked with absolute alcohol at the scleral entry site.

Trans-vitreal approach

The trans-vitreal approach is performed with a 25–30-gauge needle attached to a 5 ml syringe with a short tubing. The needle is introduced through the pars plana, 3.5–4 mm behind the limbus; the clock meridian of entry depending upon the location of tumor. The needle is guided toward the tumor with indirect ophthalmoscope or by using an operating microscope, depending upon the surgeon's preference [Fig. 6]. The needle is guided into the tumor avoiding major vessels and gentle aspiration is applied. The needle is withdrawn along the path of insertion. Localized vitreous hemorrhage is controlled by applying pressure with cotton tip applicator. Singh and Biscotti and Cohen *et al*. used trans-vitreal technique of FNAB for analysis of posterior segment tumors and concluded it to be highly diagnostic procedure with a low complication rate. The major complications associated with FNAB include needle tract seeding, hemorrhage, retinal detachment, and endophthalmitis.[7,14]

The trans-vitreal approach can also be performed with three-port pars plana vitrectomy. A vitreous separation

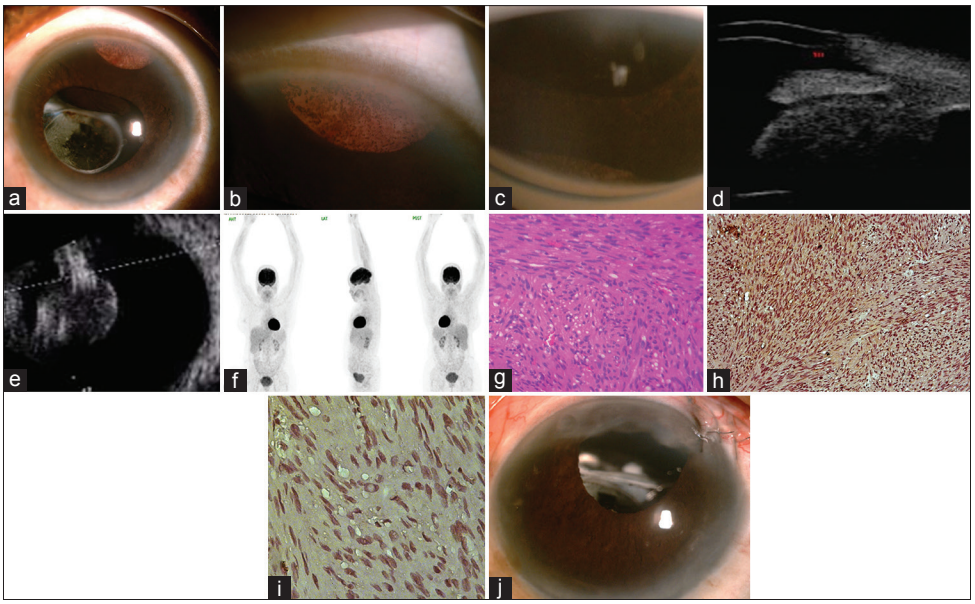

Figure 5: Partial lamellar sclerouvectomy for leiomyoma. (a) Slit-lamp photograph of the left eye reveals an orange-brown tumor behind the superior iris causing iridodialysis and extending into the pupillary axis. (b) Higher magnification view of the tumor in the area of iridodialysis shows mamillations. (c) Gonioscopy reveals no evidence of angle infiltration/tumor seeds/peripheral anterior synechiae/rubeosis iridis. (d) Ultrasound biomicroscopy reveals a well-defined retro-iridal tumor mass and supraciliary effusion. (e) Ultrasound B-scan shows a well-defined mass with high surface reflectivity and irregular internal echostructure. (f) whole body positron emission tomography scan reveals no evidence of tumor metastasis or primary active tumor. (g) Microphotograph with H and E (×40) staining of tumor reveals an amelanotic, highly cellular tumor with interlacing compact bundles of spindle cells arranged in fascicles. The cells have oval to elongated vesicular nucleus and inconspicuous nucleoli. (h) Immunohistochemical staining reveals positive staining for vimentin and (i) negative staining for S-100, consistent with diagnosis of leiomyoma. (j) Two months after surgery, iatrogenic corectopia is noticeable. Intraocular lens remains stable

is induced over the tumor and a complete vitrectomy is performed over the intended tumor site thus avoiding vitreoretinal incarceration with the needle during the biopsy. The IOP is elevated and 1.5 inch long 26-gauge needle is inserted in the tumor through the active vitrectomy port. The needle is connected to a 5 ml syringe with a silastic tube. The tissue material is aspirated from the needle and is sent for cytopathologic examination. The complications associated with the procedure are vitreous hemorrhage and an inconclusive biopsy sample.[15]

Among posterior segment tumors, FNAB is most commonly employed in reaching a diagnosis in eye with uveal melanoma, vitreoretinal lymphoma, and uveal metastasis. Retinoblastoma is a relative contraindication for FNAB because of the risk of seeding of the tumor outside the eye. The major limitations with FNAB are false negative and false positive results. Limited cellularity compromises the diagnostic potential of FNAB. A negative cytologic diagnosis should not be considered as an unequivocal proof for the absence of an intraocular malignancy.[7] In order to optimize the positive yield from FNAB, one should avoid sampling small tumors (<2.5 mm in height), avoid using thinner needles, and rinse and flush the contents of the needle and syringe using a transport medium. Having an experienced cytopathologist handle, the biopsy material is essential. Improved instrumentation and newer modalities of ocular imaging have facilitated accurate diagnosis of intraocular tumors, making FNAB a gold standard in the diagnostic evaluation of most tumor lesions. Immediately after collection of the tumor samples, the tissue is submitted to the ophthalmic pathology laboratory for further processing or is alternatively processed depending upon the tissue collected and on-site availability of cytopathologist. Nowadays, immunohistochemistry (IHC) studies are routinely used for more precise classification and immunophenotyping of tumors.

Discussion

A high diagnostic accuracy achieved with noninvasive imaging techniques is unique to the practice of ophthalmic oncologists not routinely using histologic confirmation before treating a clinically diagnosed malignancy. This combined with difficult access to intraocular tumor samples while avoiding iatrogenic vision-threatening complications and a remote possibility of systemic spread from eyes with suspected malignancy makes biopsy sampling of intraocular tumors an infrequent practice. Most intraocular tumors can nowadays be accurately diagnosed with conventional noninvasive techniques, such as indirect ophthalmoscopy, fluorescein angiography, indocyanine green angiography, enhanced depth imaging optical coherence tomography, standardized echography, ultrasound biomicroscopy, high-resolution magnetic resonance imaging, and computed tomography scans. A diagnostic accuracy of 98% can be achieved without any surgical intervention in intraocular tumors.[2] However, in atypical cases; a tissue diagnosis may be desirable. Historically, trans-scleral biopsies were initially tried and given-up for the high incidence of extra-ocular tumor spread with orbital recurrence, most probably causing an adverse effect on the tumor-related mortality.[15-17] Glasgow *et al*. have shown that the number of cells seeded when performing a direct trans-scleral biopsy is significantly higher compared with the indirect transvitreal biopsy approach.[18] Shields *et al*. had a diagnostic yield of 88%,[19] whereas Augsburger *et al*. obtained a sufficient specimen for cytopathologic diagnosis

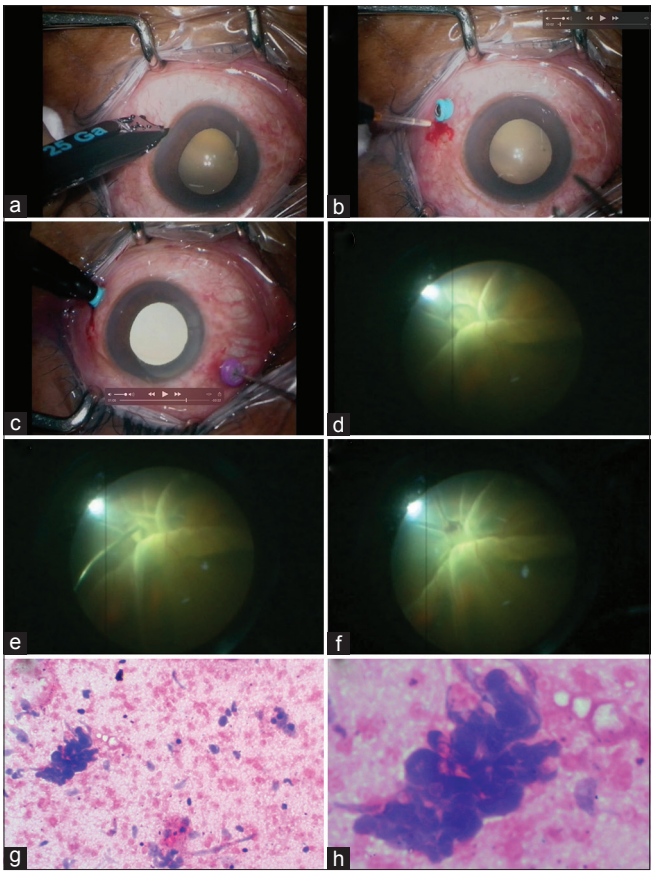

Figure 6: Trans-pars plana fine-needle aspiration biopsy with operating microscope. Using 25-gauge micro-incision vitrectomy system system (a), sclerotomy is made for chandelier light pipe (b) and another for the active instrument (c). Fundus view through the microscope with diffuse illumination (d) helps in precise placement of needle over tumor (e) and achieving homeostasis with direct visualization (f). Histopathological reveals lymphoid hyperplasia (g and h)

in 76.5% of cases.[8,20] In their study with vitreous cutter, Bechrakis *et al*. obtained sufficient tissue in all cases, which allowed a histopathologic diagnosis in 97% of cases and was also adequate for IHC studies, producing an even higher diagnostic accuracy.[15]

Most frequently used needles for intraocular biopsy are 26–30-gauge [Fig. 1]. The likelihood of insufficient sample is higher with 30-gauge needle than 24-gauge needle. A new needle with a short bevel and mm graduations has been developed.[21] Likewise, an intraocular forceps is specifically designed to retrieve tumor sample through a retinotomy (Essen forceps).[22]

A close cooperation between the ophthalmic oncologist and the ophthalmic pathologist is essential because the tumor samples are usually very small and could easily be lost during processing. Cytopathologist must interpret the cellular features within the clinical context. The role of tumor histopathology, cytogenetics, and gene expression profiling (GEP) in predicting the metastatic potential of uveal melanoma has been established. Hence, it is increasingly common to perform FNAB for prognostic purposes. Fluorescence *in situ* hybridization, single-nucleotide polymorphism array, and GEP are frequently employed to assess metastatic risk.

Even though the diagnostic accuracy of intraocular biopsy is high, limited cellularity can compromise its diagnostic potential. The traditional biopsy techniques were limited by local tumor spread, in contrast to the FNAB technique, where inadequate sampling and cytopathologic interpretation are the major limitations. In the pars-plans vitrectomy technique, a limited three-port pars plana vitrectomy is performed that allows control over IOP and potential complications such as retinal/vitreous hemorrhage, retinal break formation, and retinal detachment. Given the possibility of limited cellularity with FNAB, negative cytologic diagnosis of malignancy should not be considered unequivocal proof of absence of intraocular malignancy.

Subretinal hemorrhage localized to the site of biopsy and vitreous hemorrhage, are the most common complications. Applying pressure on the globe immediately after withdrawal of the needle is generally adequate to control the hemorrhage at biopsy site. These hemorrhages typically resolve within a few weeks. Intraocular tumor recurrence and retinal detachment are the other uncommon complications. Tumor seeding has been reported in an experimental setting after trans-vitreal and trans-scleral FNAB in enucleated tumor eyes.[18,23] The retinal break created when a subretinal tumor is biopsied transvitreally almost never leads to rhegmatogenous retinal detachment. The break is sealed by the blood clot at the biopsy site. Endophthalmitis following FNAB is extremely rare as the procedure is performed under sterile conditions.

Conclusion

Biopsy for intraocular tumors is considered a safe method of tumor sampling with regard to tumor dissemination. However, inadequate sampling and cytopathologic interpretation are the major limitations for needle aspiration techniques.

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Conflicts of interest

There are no conflicts of interest.

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