Intraocular biopsy in uveitis

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An intraocular biopsy is performed for diagnostic, prognostic and investigational purposes. Biopsies help to confirm or exclude malignancies and differentiate inflammatory from infectious processes. Histopathological analysis is the final verdict in unresponsive uveitis, atypical inflammation, metastases and masquerade syndromes. Advances and refinement of techniques in cytopathology, immunohistochemistry, microbiological and molecular biologic study offer much more than just diagnosis. They provide prognosis based on cell characteristics and are helpful in planning treatment and intervention. Many biopsy procedures have evolved to provide more safety and minimise complications thus improving the quality of specimens or samples available for analysis. The type of biopsy and technique adopted varies based on the clinical suspicion, size and location of lesions. In uveitis, a working diagnosis of intraocular inflammation is made on clinical examination and laboratory investigations and ancillary tests. Malignancy and uveitis is interlinked and masquerade syndromes are among the commonest indications for biopsy and analysis of specimen. The various types of intraocular biopsies include aqueous tap, fine needle aspiration biopsy, vitreous biopsy, iris and ciliary body, and retinochoroidal biopsy. They will be reviewed in this article with respect to current perspective

Key words: Anterior chamber paracentesis, iris biopsy, ciliary body biopsy, fine needle aspiration biopsy, retinochoroidal biopsy, uveitis, vitreous biopsy

Intraocular biopsy (IOB) is a histopathological or cytological evaluation of surgically removed samples like aqueous humour, vitreous, sub retinal fluid and tissue specimens such as iris, retina and choroidal tissues.[1] An IOB often provides a definitive diagnosis, enhances knowledge on cell characteristics, guides management, and is helpful in prognosis of the disease.

Newer methods and techniques to obtain biopsy material continue to evolve and are finding greater application in clinical decision making. Histopathological studies are now possible during early stages of the disease unlike previously when only end-stage enucleated or eviscerated specimen was available for study. Recent advances in the technique of intraocular fluid or tissue acquisition and processing allow such procedures to be used more frequently. Common indications are unresponsive uveitis, an iris or ciliary body mass, inconclusive imaging and laboratory results or masquerade syndromes.[2] Methods of IOB are anterior chamber paracentesis, iris and ciliary body biopsy, fine needle aspiration biopsy (FNAB), vitreous biopsy and retinochoroidal biopsy. We provide a review and an update of various indications of IOB and their applications.

Anterior Chamber Paracentesis

Anterior chamber (AC) paracentesis is a simple and safe technique to obtain an aqueous humour (AH) sample. In clinical practice, analysis of aqueous aspirate can provide a diagnosis in lens induced uveitis, masquerade syndrome, infectious uveitis,

endophthalmitis or when retinal examination is not possible due to vitreous inflammation.[3] The utility of aqueous humour analysis for the diagnosis of posterior uveitis has been reported by Rothova *et al*.[4] The easy accessibility of AH and safety of AC paracentesis compared to other biopsy procedures has stemmed a lot of research in both anterior and posterior segment pathology.

Technique

AC paracentesis can be performed in the out-patient clinic under topical anaesthesia using proparacaine 0.5% and aseptic precautions (0.5% povidone iodine). It can be performed using a slit lamp, or without slit lamp (if patient uncooperative/ unable to sit) or an operating microscope but lens injury is minimised when performed with the patient in supine position. 30-G needle mounted on a tuberculin syringe is used to enter AC near the inferotemporal limbus. 0.1-0.2 ml of aqueous is aspirated. The bevel of the needle faces the cornea to prevent iris incarceration. A partial thickness corneal incision is self-sealing and reduces risk of lens injury. The needle is withdrawn while placing a sterile cotton-tipped applicator at point of entry. Gentle pressure is applied at site of entry for 10-20 seconds. The eye is patched after applying antibiotic eye drops. Reassessment is done after half an hour to ensure reformation of AC and to exclude hyphema.[5] [Fig. 1].

A newer technique, using an aqueous pipette for sample collection has been described as an effective way for manipulation

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and aliquoting. It consists of a short 30 gauge needle attached to plastic tubing which is further connected to a suction and infusion bulb. Squeezing the bulb creates vacuum and with release of pressure aqueous collects in the disposable pipette. The main advantage with this technique is that it produces no dead space and hence can be used in shallow anterior chamber.[6]

Processing of the specimen

The Cytospin technique has been reported to maximise cell yield. Cytospin slides containing up to 0.4 ml of fluid are added to a sample delivery chamber and centrifuged by spinning at 1800 rpm for 2 minutes. The slides were removed from the chamber and fixed with formalin or 70% alcohol and stained with haematoxylin and eosin.[7]

Following paracentesis, a portion is used for direct smear, culture and polymerase chain reaction (PCR) for suspected microbes. The remaining fluid undergoes cytospining for cytopathological examination.

Applications of aqueous humour analysis in diagnosis and research

- Lens induced uveitis: Phacoanaphylactic endophthalmitis can be diagnosed based on histopathological features such as chronic inflammatory infiltrate, lens laden macrophages around the retained lens fragment. Western blot test using antibodies to detect lens- specific proteins in aqueous humour has been described recently as a sensitive and specific method in traumatic uveitis[8]
- Western blotting to detect local antibody to specific microorganisms and Goldmann- Witmer coefficient (GWC) to quantify antibody production
- Aqueous PCR identifies DNA (deoxyribonucleic acid) of infectious organisms and can confirm diagnosis. A favourable response to changed management following PCR was noted in 38% of patients.[9] Aqueous PCR test is particularly useful in immunosuppression as presentation can be atypical or when dual positivity is suspected, where a prompt sampling and rapid test is required. Reliable positive PCR results on aqueous humour analysis in the detection of posterior uveitis due to tuberculosis have been reported by Rao *et al*.[10] A positive correlation between the size of retinochoroiditis and positive aqueous PCR was found. Combining PCR, GWC and Western blot technique increased the sensitivity by up to 97%[11]
- Increased levels of TGF beta- 2 in the AH could indicate an increased risk of secondary glaucoma in uveitis associated with juvenile idiopathic arthritis

- Levels of IL-10 (interleukin - 10) alone from an AH sample when more than 50 pg/ml can be can be a marker of intraocular lymphoma.[12,13] Cytokines, metalloproteinases and chemokines detected by ELISA in the AH are being studied for their diagnostic and prognostic significance as pro- or anti- inflammatory mediators in uveitis.[14]

Iris and Ciliary Body Biopsy

These are excisional biopsy procedures indicated in neoplasms, inflammations, metastasis and cysts. In the anterior segment, juvenile xanthogranuloma and intraocular lymphoma can masquerade as uveitis.[15]

Technique

Iris biopsy or endo-iridial biopsy is done with a Kelly's Descemet membrane punch. The trabeculectomy punch can be used to access within 7 mm of the corneal incision. It is easier to perform and cheaper but the punch may not provide samples in small lesions.[16]

Iridectomy: Iridectomy provides enough diagnostic material in 90% of the patients.[17] Resection of lesion with a surrounding 2 mm frill of healthy tissue from the tumour margins through a limbal or corneoscleral route is indicated when vision is threatened due to rapidly growing tumour, pupillary encroachment, and as an eye salvaging procedure in malignancy. Pre-procedural pupillary miosis prevents pupillary distortion and photophobia. Surgical iridectomy with a vitrectomy cutter is used to obtain a larger specimen has been described by Ghanem *et al*.[18] Recently bimanual manipulation with 23G scissors and forceps introduced into the anterior chamber has been described. The technique described by Chronopolus *et al*. uses a specially designed Essen -23G biopsy forceps used in pigmented tumours. Two corneal incisions are made, one for introducing the forceps into the anterior chamber and a second for the use of 23G scissors. The specimen is removed from the anterior chamber and fixed.[19] Minimal bleeding and post procedure mydriasis were the complications reported.[20]

It is performed under general anaesthesia and is indicated in ciliary body tumours and iris tumours involving the angle. A larger incision than iridectomy is required to prevent the tumour from touching edges of normal tissue during removal. Sympathetic ophthalmia has been reported as a complication of this procedure.[21]

Micro-incision iris biopsy or "Finger iridectomy technique" is an effective way of obtaining tissue from an iris tumour and provides 98% results from cytological analysis.[22] It is least invasive and is performed through a self-sealing corneal or limbal incision. This technique uses a single 25-G aspiration

Figure 1: Showing the procedure of anterior chamber paracentesis done under aseptic precautions
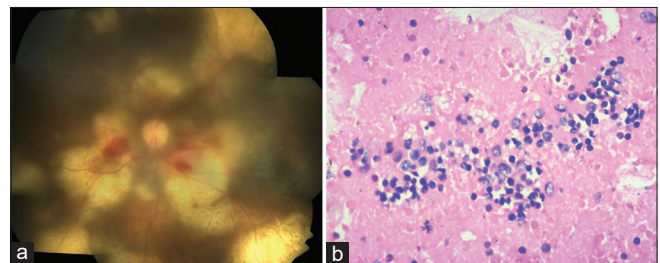
Figure 2: (a) - Montage fundus photograph of a case of primary intraocular lymphoma following FNAB, showing multiple choroidal infiltrates with vitreous haemorrhage due to FNAB. (b) - Microphotograph of a FNAB specimen showing multiple large lymphoid cells with high nucleo-cytoplasmic ratio seen in a necrotic background (Haematoxylin and eosin x 200)

cutter probe to perform multiple iridectomy biopsies through a single 1-mm incision under 1% sodium hyaluronate 1%. It differs from the Ghanem technique in that there is no scleral phacoemulsification incision or irrigation.[21]

It is a small incision biopsy technique of anterior segment tumours where large samples can be obtained for histopathological evaluation. Secondary glaucoma due to retained viscoelastics has been reported.[23]

A 25-G aspiration cutter assisted anterior chamber biopsy technique and trans-corneal aspiration using fine cannula are newer procedures. Adequate biopsy specimen is obtained in 100% of the patients.[24]

Iris and ciliary body biopsy specimens can also be obtained by fine needle aspiration biopsy (FNAB). This procedure will be discussed subsequently in the review under FNAB.

Common complications in all iris and ciliary body biopsies are hyphaema, hypotony, lens injury, seeding of episcleral tissue and endophthalmitis. Iris tissues are extremely fragile. They should be quickly transferred to the laboratory in the suitable preservative required for storage and transportation. The specimen is transferred on ice or ice pack for flow cytometry, culture and PCR studies. For cytological analysis, culture medium or a fixative solution like HOPE (Hepes-glutamic acid buffer-mediated organic solvent protection effect) solution is used. HOPE solution not only preserves cytological details, but also the immunoreactivity and nucleic acids.[25]

Vitreous Biopsy

The vitreous is an acellular matrix and the presence of cells or inflammatory mediators suggests uveitis or neoplastic processes involving the ciliary body, choroid, retina or optic nerve. It provides more information than AC paracentesis and has fewer complications than retinochoroidal biopsy. The specific indications of vitreous biopsy are atypical posterior uveitis when other tests are inconclusive, unresponsive and persistent vitreous inflammation such as in primary vitreoretinal lymphomas (PVRL), vision threatening uveitis or in identification of the causative agent in exogenous and endogenous endophthalmitis.[26]

Vitreous biopsy is best done by a vitreoretinal surgeon and can be performed under local anaesthesia (LA). A sample can be obtained by vitreous tap (needle aspiration) or by pars plana vitrectomy (PPV). In needle aspiration, a short 26-gauge needle mounted on a syringe is inserted 3.5-4 mm from the limbus and vitreous is aspirated. Valved cannulae are preferred and the sclerotomies are sutured. Complications include lens injury, vitreous haemorrhage, retinal detachment and endophthalmitis. In PPV, the detached vitreous is aspirated from the cutter and not from the cavity thus minimising traction. An increased diagnostic yield, removal of inflammatory debris/pathogen load/neoplastic cells, better drug penetration, visualisation of the fundus and an improvement in visual acuity makes PPV a preferred mode of obtaining material for vitreous biopsy.

Advances in vitreous biopsy techniques

Initially vitreous core biopsy was performed through a single sclerotomy as a diagnostic procedure in endophthalmitis.[27] It was modified to use a 3-way stopcock for manual aspiration then to a pneumovitrector that has an aspiration and cutting system with an infusion line for fluid-gas exchange into the vitreous cavity. 20-G, 23-G, 25-G have been replaced by 27-G cutters as the cutting speed may degrade the quality of the sample.

Subsequent innovations include trocar – cannula system for insertion of instruments and 27-G instrumentation.[28] Zhao et al. have described a technique done under air infusion using 23-G vitrectomy which harvests undiluted vitreous, increases biopsy yield and decreases post procedure inflammation.[29] The advantage of performing vitreous and retinal biopsy using 25-G transconjunctival sutureless vitrectomy and microcannulas under topical anaesthesia has been reported. The advantages are that 25 gauge procedures cause less inflammation and induce less ocular trauma allowing quicker visual rehabilitation.[30]

Diagnostic vitrectomy

A three port PPV has an overall yield of 12.4-64.3% and is preferable for diagnostic vitrectomy.[31] Manual vitreous aspiration is performed at the beginning of the procedure to obtain 0.1 ml of undiluted vitreous. A cutting rate of 1000/minute is used. Infusion is then opened and a total vitrectomy is performed. After the undiluted specimen is collected, a second syringe is attached to the vitrector to collect 3-10 ml of diluted vitreous sample. Maximum cellularity is present in the cortex in inflammation. The vitrector cassette, undiluted vitreous specimen and subsequent washings from total vitrectomy should be sent for analysis. The sample has to be transferred at the earliest to avoid necrosis of the inflammatory or neoplastic cells [Video 1].

Analysis of vitreous

Ideally, the vitreous specimen is divided into three samples. One for cytopathological examination, the second portion is to be frozen for immunohistochemistry and molecular analysis and the third for culture and PCR for microorganisms.

The undiluted specimen is used for culture, PCR and immunohistochemical study. Immunohistochemistry uses immunofluorescence (fluorescence labelled) or immunoperoxidase (enzyme labelled) to detect antigens. Flow cytometry does cell sorting based on fluorescence activated characteristics.

Processing of the specimen

Diluted specimen is used for cytologic analysis (after centrifugation) and culture. Centrifuging the vitreous sample at 500 rotations per minute at room temperature for 6-8 minutes significantly improves the quality of the sample available for cytopathological evaluation. Portions of this cytospin specimen are air-dried and stained with haematoxylin and eosin or Giemsa stain which is fixed in 4% formalin overnight, dehydrated and embedded in paraffin blocks for sectioning.

Cytopathology would detect cells and in combination with immunohistochemistry and flow cytometry would offer cell classification which is very useful in providing cell characteristics.

Research in vitreous biopsy

Recently, it has been reported that preparation of cell block and analysis is superior to conventional smear cytology in vitreoretinal lymphoma. Small tissue fragments that are retrieved from the block have an increased yield for tissue sectioning.[32]

Vitreous biopsy in uveitis

Results of vitreous biopsy have been proven to be highly accurate and can alter the management of uveitis. In infectious uveitis, stains and smears are useful for initial rapid diagnosis but only 66% can be proven by culture. Cultures need to be performed on diluted and undiluted samples as it increases sensitivity by 57.4%. Sensitivity of vitreous culture is 50% higher than with aqueous.[33] Culture methods were superior in fungal endophthalmitis which grew organisms in 80% of

patients and was least in endogenous bacterial endophthalmitis by both methods and confirmatory in 17% of patients.[34] Combining PCR and culture results is often confirmatory.

Manku *et al*. have reported that PCR is more sensitive over microbiological culture in viral uveitis and could identify the causative agent in 87.5% of patients.[35] In diagnostic uncertainty, PCR is very useful in HSV, VZV, CMV, EBV and toxoplasmosis. The volume available for testing is higher with vitreous sample and multiplex PCR is particularly helpful in immunosuppressed state when dual pathology may exist.

Vitreous biopsy in endophthalmitis

The sensitivity of PCR in the detection of organisms in bacterial endophthalmitis was 66% compared to 34% with culture. False positive rates with PCR on vitreous sample were 3.4% based on reports by Joseph *et al*.[36] In fungal endophthalmitis, candida and aspergillus are best detected on culture. In chronic postoperative endophthalmitis, PCR is superior to culture due to less organism load and slow growth. It has been reported to be 92% by PCR and 6% with culture.[37] Toxoplasmosis can resemble viral retinitis especially in immunosuppression.[38] Vitreous analysis for toxoplasma DNA using PCR or GWC can be useful.[39] GWC compares intraocular antibody production with serum antibody levels and ratio greater than 1 being abnormal and ratios greater than 2 suggesting significant disease.[40] Combined use of GWC and PCR are recommended as positive reactions to both occur during different stages of the disease.[41] In tubercular uveitis, microbiological and molecular biologic tests on the vitreous sample are important for diagnosis and treatment.[42]

The role of cytopathological findings such as non caseating granulomas and multinucleated giant cells consistent with sarcoid uveitis have been reported in vitreous specimens in those with suspected sarcoid associated uveitis.[43] Cytokines are upregulated in ocular sarcoidosis and their levels correlate with the severity of cystoid macular oedema.[44]

In the case of autoimmune uveitis, pro-inflammatory cytokine such as interleukin 1 (IL 1), IL2, IL6, IFN-y and tumour necrosis factor (TNF) alpha levels are higher.

Vitreous biopsy in the diagnosis of intraocular lymphoma

Vitritis occurs in 60% of intraocular lymphoma and is the commonest ophthalmic clinical presentation.[45] Other presentations are chorioretinal infiltrates in 25% and panuveitis in 15%.[46] Prior treatment of lymphoma with corticosteroids can change the phenotype of cells and it is ideal to taper steroids prior to vitreous sampling. A negative biopsy needs to be repeated in strong clinical suspicion. A retinochoroidal biopsy is indicated for further confirmation. Adequate amounts (2 ml) of vitreous needs to be analysed as soon as possible or it needs to be fixed immediately to preserve cells. Lymphoma cells have scanty basophilic cytoplasm, prominent nucleoli and high nuclear cytoplasmic ratio. Haematoxylin and eosin stain or Giemsa stain can demonstrate lymphoma cells. Immunohistochemistry is used to identify specific markers such as CD22 (cluster of differentiation), CD20, CD19.[47] Flow cytometry demonstrates monoclonality, surface markers and surface antibodies.[48] High levels of IL10 and Il 10: Il 6 ratio greater than 1 is highly suggestive of IOL. TCR gene rearrangements occur in the rarer T cell lymphoma.

Vitreous biopsy in Pan-uveitis

Seasonal Hyper Acute Pan-Uveitis (SHAPU) a form of uveitis has been described in which the etiology remains an enigma. Bacteria, viruses, immune response and recently moths have been reported as possible causes based on reports from vitreous biopsy specimens.[49]

Fine Needle Aspiration Biopsy (FNAB)

In uveitis, FNAB is indicated in suspected sub retinal abscesses, tuberculoma, inconclusive uveitis and masquerade syndromes.[50] The risk of complications is much less with FNAB than with retinochoroidal biopsy or PPV. Intraocular haemorrhage that may occur usually settles spontaneously. Very rare complications are retinal detachment, dissemination of malignant cells and endophthalmitis. Limited material obtained despite good technique could suggest cohesive cells in the aspirate suggestive of benign nature of the tumour. Thinner needles result in low yields and needles have to be flushed into transport medium even during dry taps. The role of pathologists are pivotal and their presence in the operating room will permit an immediate histopathological study, prevent repeated biopsy attempts and decrease false negatives. Sampling errors need to be considered in negative specimens. Aspirate obtained from outside the area of pathology can be misleading and a negative cytological result does not always prove absence of malignancy if clinical indicators suggest otherwise.

FNAB can be performed for the anterior and posterior segment. The site of needle placement is critical to the type of aspirate and the diagnosis. An open biopsy may be used in the anterior segment but not anymore in the posterior segment where vitrectomy based biopsy procedures are more popular.[51] The entry site is opposite to the location of the lesion. A 60-80 degree angled bevelled tip keeping the needle parallel to ocular tissues reduces trauma.

Fine needle aspiration biopsy of the anterior segment

FNAB provides a diagnostic yield in 99% of patients in phacoanaphylactic uveitis.[52] An inferior or temporal corneal entry is made just inside the limbus. A 26-G needle is attached to a polyethylene tube which is further connected to a syringe. The 12-20 mm long tubing provides stability to the needle. The needle is passed within the mass and cells are aspirated by manoeuvring the needle tip. Stromal hydration and patching are performed at the entry site. Complications are hyphaema, raised intraocular pressure, lens injury, infection and wound leak. The limitation is that only a small sample can be obtained.

Surface contact vacuum is a newer technique performed at the slit-lamp. A needle attached to a tuberculin syringe is used. The needle is used bevel down without tubing and 0.5 ml of aspirate is obtained in juvenile xanthogranuloma and intraocular lymphoma masquerading as uveitis.[53]

Fine-needle aspiration biopsy of posterior segment

FNAB requires a clear view of the retina, and is difficult to perform in opaque media. The procedure offers 88-95% safety and reliability.[54] The clinical diagnosis correlated with cytological diagnosis in 80% of lymphomas.[55]

- In the trans-scleral approach, a scleral flap of 3 mm and 80% depth is made. A 26-30G needle attached to tubing is inserted through the scleral bed and a sample is obtained. The scleral flap is closed. Cryotherapy is applied to the entry site
- The trans-vitreal approach can be performed by two methods using the pars plana route. A 26-30G needle attached to a short tubing and 5 ml syringe is passed. The needle is passed into the tumour and sample is withdrawn. The second method uses a three port PPV. A 26 G needle is connected to a 10 ml syringe through a 12-20 mm long plastic tube. The tubing prevents movement when suction is used for aspiration. The

needle is inserted through the pars plana, advanced into the lesion, aspiration performed and withdrawn. Both procedures should be done under an operating microscope [Fig. 2].

Retinochoroidal Biopsy

Retinochoroidal (RC) biopsy is often the last resort in evaluation of patients because of the risk of complications and the complex surgical procedure involved. It is performed in unresponsive, vision threatening uveitis when malignancy is suspected, in atypical uveitis when vitreous biopsy and other investigations are negative or when the disease is confined to the sensory retina, RPE or choroid and in retinal or sub retinal mass/infiltrates when previous imaging and laboratory investigations were inconclusive. The commonest indications are lymphomas with predominant sub retinal infiltrates with little or no vitreous involvement.

Technique

RC biopsy can be performed through one of the following routes[56]:

- External approach or transscleral approach
- Internal approach which may be transvitreal or an FNAB.

It can be done under local or general anaesthesia and the approach depends on the location to be biopsied. The trans-scleral approach is rarely used as it has a high risk of suprachoroidal haemorrhage and extrascleral seeding. It is performed if at all, only in those lesions that are anterior to the equator or around the ora serrata where direct visualisation is possible to control intraocular bleeding with diathermy or internal tamponade. The biopsy is obtained by making a full thickness sclera flap and incising the choroid with a sharp blade.

The transvitreal approach is safer. A standard PPV is performed and the area of choroid to be excised is delineated with a diathermy or laser. A vertical segmentation scissors is used to cut the retina and choroid and the specimen is removed with forceps through a sclerotomy. Minimally invasive 25-G trans-vitreal retinochoroidal (TVRC) biopsy to obtain larger samples for histopathological and immunohistochemical study has been described in selected situations.[57]

A transvitreal FNAB is performed using a 26 G needle connected to a 10 ml syringe through a plastic tube. The tubing prevents movement when suction is used for aspiration. The needle is inserted through the pars plana, advanced into the lesion, aspiration performed and withdrawn. This procedure should be done under an operating microscope or indirect ophthalmoscope.

In infectious retinitis, a RC biopsy is performed in consultation with the ocular pathologist and microbiologist for decisive tests based on the suspected organism. Specimen is excised at the junction of the involved and uninvolved retina as the margins have high activity.[58] Central areas are avoided as they are likely to be necrotic. The biopsy specimen needs to be immediately divided and fixed in formalin or gluteraldehyde for histopathology and electron microscopy respectively. A portion of the tissue is frozen for molecular biologic study. Detection of aspergillus endophthalmitis following RC biopsy has been reported.[59]

The incidence of uveitis is 2-2.5% in vitreoretinal lymphoma.[60] In lymphoma, patients presenting with uveitis, definitive diagnosis of RC biopsy was made in 59% and excluded in 31% of patients.[61] The rate of diagnosis of PVRL following RC biopsy is high because the sub RPE space is the primary site of involvement. Specimens must be taken from deeper parts of the lesion and near the choriocapillaries as tumour cells are found mainly in perivascular locations. Superficial and sub retinal lesions are necrotic and a specimen from these locations would give a false negative result. Laser capture microdissection technique by isolating lymphoid cells from tissues sections increases sensitivity and specificity of PCR from 60% to 100%.[62] Recently, chromosomal tests on RC specimens of PVRL such as detection of mutations in primary response gene 88 and immunoglobulin heavy chain rearrangements have been described by Dawson *et al*.[63]

Conclusion

Intraocular Biopsy results have high predictive values in uveitis and in tumours. In uveitis, apart from establishing a diagnosis they provide insights into the pathogenesis of the disease process. Intraocular biopsies should be sent with care either fresh or in fixatives. Specimen yield, handling and processing is integral to a reliable biopsy report.

Conflicts of interest

There are no conflicts of interest.

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