Histopathological, Immunohistochemical and Molecular Biologic Study of an Enucleated Specimen of a Case of Eales' Disease.

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Brief report

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Abstract

Eales' disease is a retinal vasculitis characterized by retinal inflammation, ischemia, and neovascularization. Exact pathogenesis of this disease is yet to be found out. We present a 29-year-old male, diagnosed as Eales' disease in both eyes with persistent intraocular inflammation. Enucleation of the pthisical right eye was subjected for histopathological examination immunohistochemistry and molecular biologic study for mycobacterial tuberculosis DNA. Our study showed that Eales disease is a T cell mediated disease which is triggered by mycobacterial TB DNA

Introduction:

Eales' disease is a retinal vasculitis predominantly affecting the peripheral retina of young and otherwise healthy adults.[1] Etiopathogenesis of this disease is still not clear.[1] Recent molecular biologics studies had shown mycobacterium tuberculosis DNA in Eales disease specimens.[2] Histopathological study of Eales disease eye has rarely been done. We report histopathologic, immunohistochemistry (IHC) and molecular biologic study of an enucleated specimen of a case of Eales' disease.

Case Report:

A 29-year-old Asian Indian male was diagnosed as Eales' disease in the left eye and was referred to the uvea clinic of our tertiary eye care center in south India for persistent inflammation in the left eye. He was treated with oral steroid and had pan-retinal laser photocoagulation of neovascularisation of retina. He had poor vision in the right eye since 10 years.

On examination, his best corrected visual acuity was no perception light in the right eye and 20/200; N36 in the left eye. Right eye was phthisical with pupillary membrane, peripheral anterior and posterior synechiae, complicated cataract and low intraocular pressure.

Anterior segment of left eye was normal. Indirect ophthlomoscopy of the fundus showed media haze at the posterior pole, a pale optic disc with attenuated and sclerosed vessels. There was few retinal hemorrhages along with collaterals temporal to the macula. Mid-peripheral retina showed laser photocoagulation scars [Fig. 1A]. Fundus fluorescein angiography in the arterio-venous phase showed disc leakage with active vasculitis temporal to fovea [Fig. 1B]. Ischemic areas were also noted temporal to macula. Fundus lesions were suggestive of active Eales' disease in the left eye. The patient was investigated for causes of retinal vasculitis. Laboratory investigations for toxoplasma, syphilis, sarcoidosis and collagen diseases were negative. The right eye was enucleated for cosmetic reasons. The eyeball was fixed in 10% neutral buffered formalin, sectioned axially and subjected to processing for paraffin embedding. Serial sections were stained with Hematoxylin and Eosin, Masson's trichrome stain for collagen and Ziehl-Neelsen stain(ZN) for acid fast bacilli. IHCs was performed for multiple markers which included glial fibrillary acidic protein (GFAP), CD45, CD68, CD3, CD4, CD8, CD20, CD138, MPO, IgG and IgG4.

Histopathology revealed a phthisic eyeball with a thick epiretinal membrane and retinal detachment. The epiretinal membrane was densely collagenized and scarred, relatively avascular with sclerotic vessels reflecting chronicity and flanked above and below by metaplastic bone. A think band of dense fibrillary gliosis was seen in the adherent retina (Fig. 2A). Inflammation was seen forming a small aggregate beneath the ciliary body. It had an admixture of lymphocytes, histiocytes with elongated nuclei and epithelioid morphology forming loose clusters, admixed with lymphocytes and few polymorphs. The lymphoid cells were CD3 + T cells with scant to absent CD20 + B cells. The T cells were CD8 + cytotoxic cells. No CD4 + T cells or plasma cells were seen. IgG4 was negative. The inflammatory focus had elicited a fibrillary gliosis and collagenization entrapping thin vascular channels (Fig. 3A-H). ZN stain for acid fast bacilli was negative.

DNA was extracted from the paraffin section and nested PCR targeting MPB64 gene and IS6110 region of MTB genome were found to be positive [Fig. 5A]. Real time PCR showed 3,460 of copies of MTB DNA [Fig. 5B].

Patient was put on four drug antitubercular treatment with oral Prednisolone 60 mg per day which was gradually tapered over 6 weeks. On last follow up, there was complete resolution of vasculitis. However, there was no improvement of vision in OS due to macular ischaemia.

Discussion:

Eales disease is a retinal vasculitis characterized by retinal inflammation, ischaemia and neovascularization.[1] There were only few histopathologic studies on Eales' disease eye performed.[3] PUBMED search did not show any IHCs studies of Eales' diseases so far. Our study showed cytotoxic Tcells in the eye ball specimen of Eales disease. T cell mediated immunologic reaction has been found in Behcet's disease, [4] Vogt Koyanagi Harada disease[5] and sympathetic ophthalmia.[6] Our study indicates in active stage of Eales disease, immunomodulatory therapy with T cell inhibitors may be effective. The disease thought to be an immunologic reaction that can be triggered by mycobacterium tuberculosis (MTB) DNA. We had earlier reported that significant lymphocytic infiltrations were seen in the vitreous specimens in Eales disease.[1] Molecular biologic study of the vitreous sample[7] as well as epiretinal membrane[8] of Eales' disease by PCR showed MTB DNA. We had also found earlier TB DNA in the paraffin retrieval sections of enucleated globes in Eales disease. [3]

Our present study showed for the first time that Eales' disease is associated with MTB by nested and real time PCR of the enucleated specimen correlated with histology and IHCs. IHC finding predominantly showed CD8 + T cells lymphocytic infiltration in the enucleated specimen.

We conclude that Eales' disease is a cytotoxic T cell (CD8) mediated process which is triggered by mycobacterial MTB DNA.

Declarations

Ethics approval and consent to participate: Ethics approval: Not applicable; Patient's consent was duly obtained prior to any imaging.

Consent for publication: Not applicable

Availability of data and materials: Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

Competing interests: The authors declare that they have no competing interests in this study.

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Figures

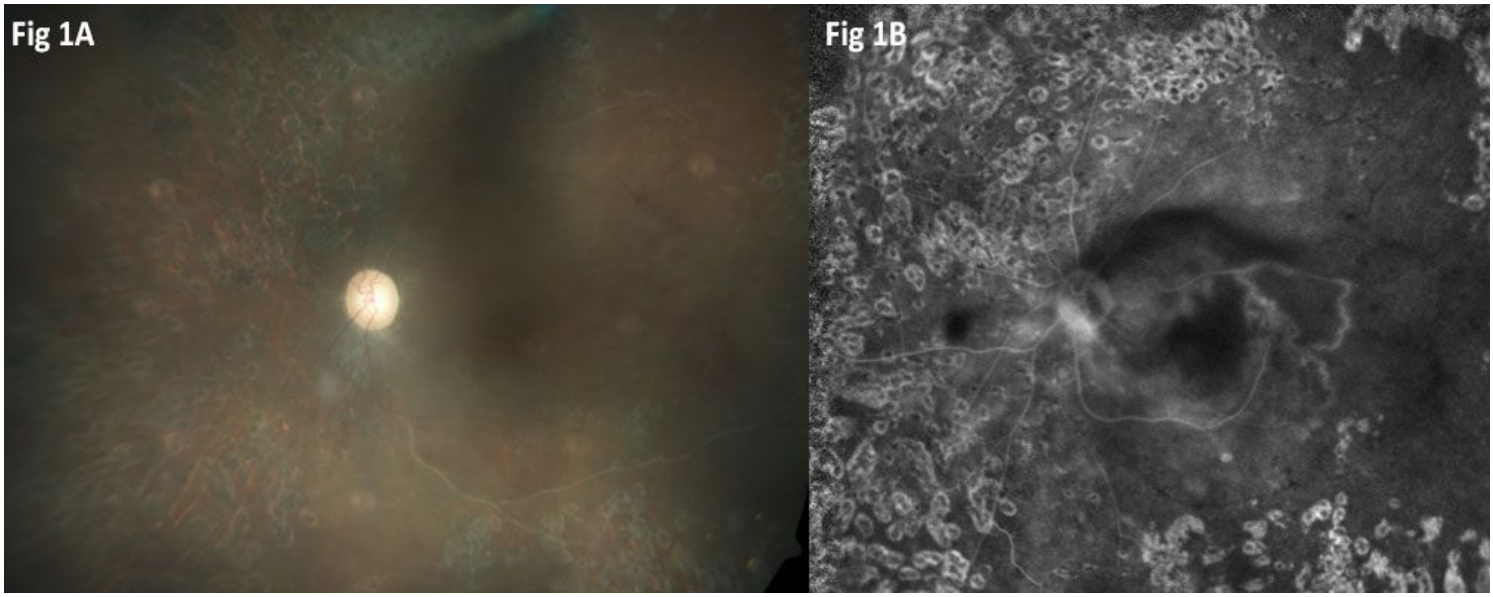


Figure 1

1A: Clinical photograph of left eye fundus showing media haze at the posterior pole, a pale optic disc with attenuated and sclerosed vessels. There are few retinal hemorrhages along with collaterals temporal to the macula. Mid-peripheral retina shows laser photocoagulation scars. 1B: Fundus fluorescein angiography in the arterio-venous phase showing disc leakage with active vasculitis temporal to fovea. Ischemic areas are also noted temporal to macula.

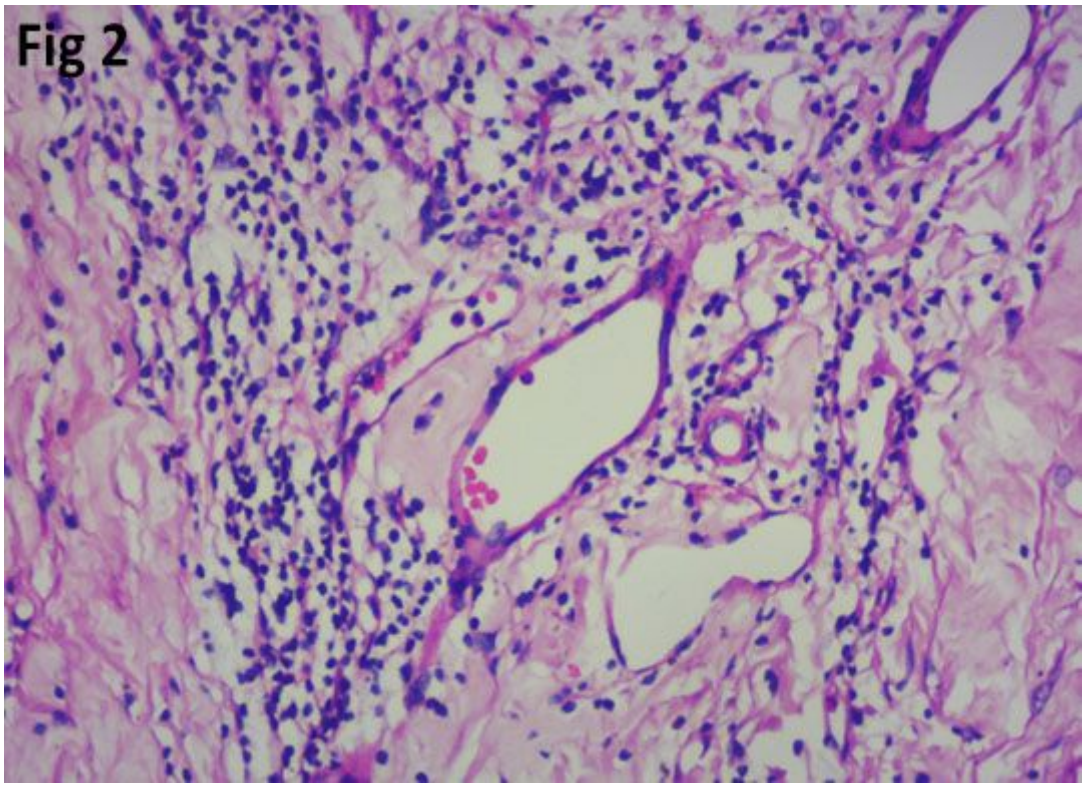


Figure 2

A: Histopathology section shows neovascularization into vitreous cavity with presence of lymphocytes in the inner vascular areas as well as perivascular spaces.

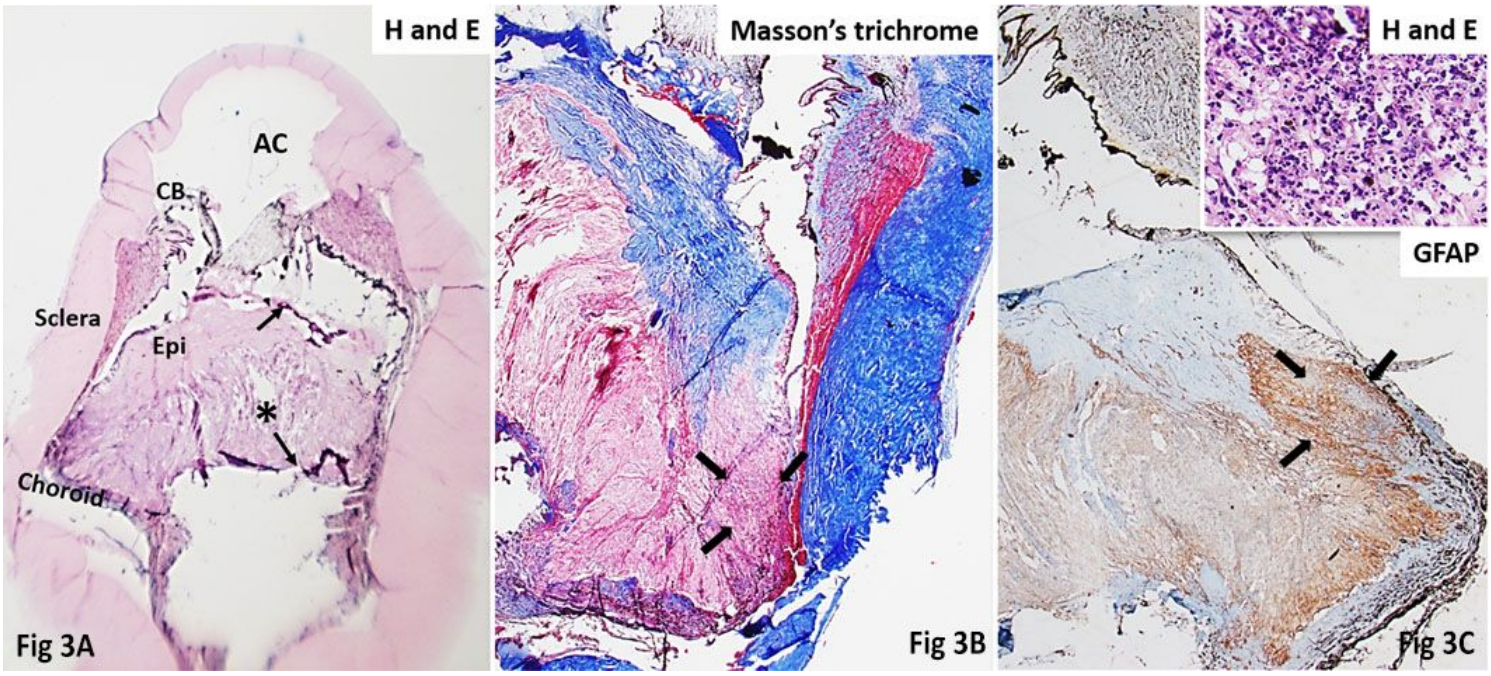


Figure 3

Scanner view of eyeball showing pthisis bulbi[A]. A thick epiretinal membrane seen densely fibrotic, scarred, relatively avascular and flanked above and below by metaplastic bone (B). The retina is detached with dense fibrillary gliosis along epiretinal surface. [C] Note focus of inflammation close to ciliary body [arrows B; C inset] surrounded by gliosis [C, arrows] [A: H&Ex8; B: Masson trichromex40; GFAPx40] Note: The designations employed and the presentation of the material on this map do not imply the expression of any opinion whatsoever on the part of Research Square concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. This map has been provided by the authors.

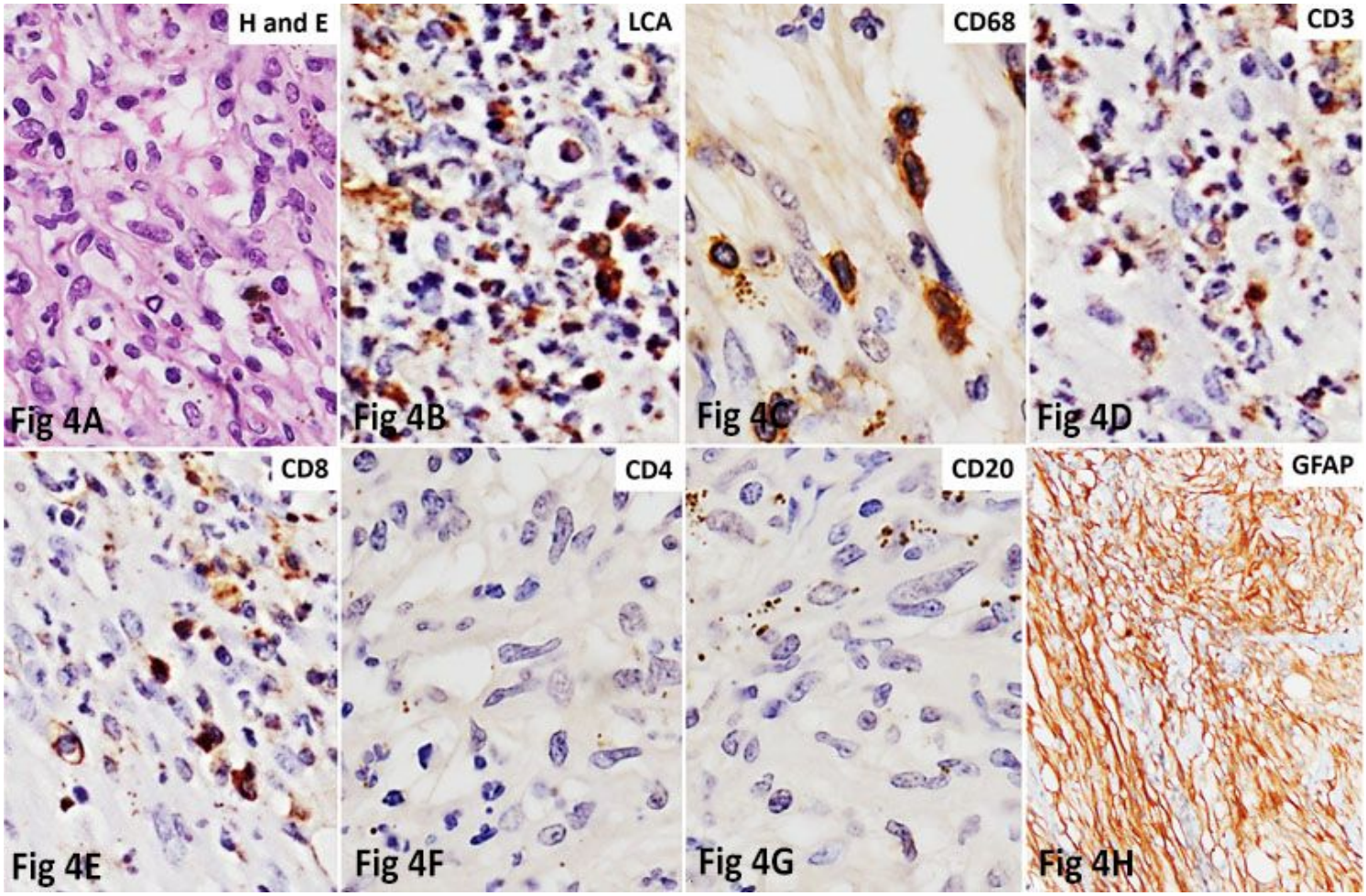
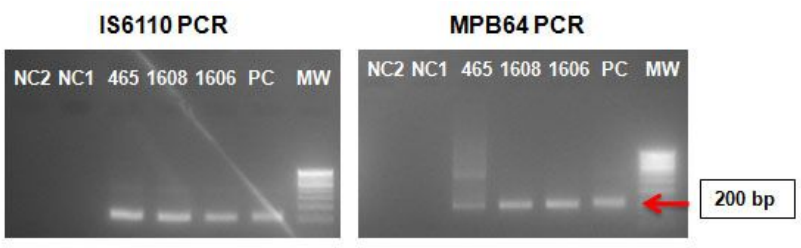


Figure 4

Higher magnification of inflammatory focus shows collection of epithelioid cells forming an ill formed granuloma [A]. These are admixed with lymphoid cells labelled by CD45(B). The epithelioid histiocytes are CD68+ [C] and lymphoid cells are predominantly CD3+ [D], CD8+ cytotoxic in phenotype [E] without CD4+ T cells [F], CD20+ B [G], eliciting a fibrillary glial reaction [H]. [A:H&Ex160; B:LCA-CD45x160; C:CD68x240; D:CD3x160; E:CD8x160; F:CD4x200; G:Cd8x200; H: GFAPx80]



NC2: Negative control (reagent control)
NC1: Negative control (transfer control)
465: DNA extracted from paraffin section of the enucleated eye
Positive for MPB64 and IS6100 PCR
1608 & 1606 : DNA extracted from clinical; sample
PC: Positive control DNA (H37RV)
MW : Molecular weight ladder (100 bp)

Fig 5A

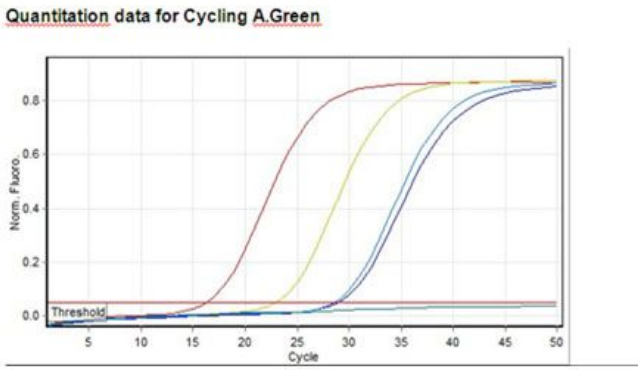


Fig 5B

Figure 5

A: Nested PCR for DNA extracted from paraffin section of the enucleated eye positive for MPB64 and IS6110. B: Real time PCR of DNS from paraffin section of enucleated globe (Blue) showing 3460 copies.