

In Vivo Confocal Microscopy Features In Post COVID19 Fungal Endophthalmitis In Type 2 Diabetes

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Original research

Keywords: SARS-CoV2, COVID-19, confocal microscopy, ocular candidiasis, fungal endophthalmitis, Molecular diagnostic methods, PCR, Candida Tropicalis, Corneal nerve fiber analysis

Posted Date: May 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-519477/v1>

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Abstract

Introduction: Corona virus disease-19 (COVID-19) infection with aggressive treatment has resulted in immunosuppression, and co-existing comorbidities that have made patients vulnerable to secondary infections including fungal infections.

Purpose: We describe the clinical presentation, various diagnostic methods including in-vivo confocal microscopy features, management and clinical outcomes in Post COVID-19 fungal endophthalmitis in type II diabetics.

Methods: Prospective, interventional study on two patients who recovered from COVID-19 who developed endogenous endophthalmitis presented to a tertiary eye care centre in South India.

Results: Fungal infection was diagnosed based on clinical features, confocal microscopy, molecular diagnostic methods and culture methods. Two patients were treated with topical, intravitreal and systemic antifungal therapy.

Conclusion: We report for the first time two cases of post COVID-19 fungal endophthalmitis in two diabetic patients with confocal features. High index of suspicion, early diagnosis, and appropriate management can improve the visual outcome in post COVID-19 fungal endophthalmitis cases.

Introduction

Corona virus disease-19 (COVID-19), first reported in Wuhan in China in December 2019 ¹ The COVID-19 manifestations can range from asymptomatic presentation to severe respiratory distress in severe cases. It affects all parts of the body including the eye. Ocular manifestations include involvement from lid to optic nerve. Different ocular manifestations occur during different phases of the disease.² Ocular manifestations may result from systemic COVID 19 infection, adverse reactions to treatment and various opportunistic infections. Sen M et al have reported mucormycosis in high risk COVID -19 patients who are recovering from COVID- 19 infection.³ Here we are reporting two cases of post COVID- 19 fungal infection in type II diabetes mellitus presenting in the form of unilateral endogenous endophthalmitis with clinical course and confocal microscopy features for the first time in published medical literature. Changes in corneal nerves were analysed using CCmetric analysis software

Case 1

A 54 year Asian Indian male, a known case of type 2 diabetes mellitus who was on oral hypoglycemic agents developed severe COVID 19 infection. At the local hospital nasopharyngeal swab for *Reverse Transcription Polymerase Chain Reaction* (RT PCR) for severe acute respiratory syndrome corona virus-2 (SARS CoV-2) confirmed the diagnosis of COVID 19 infection for which he was treated with (Inj. Remdesivir(100mg BD), T. Dexamethasone(6 mg), Inj Tocilizumab 400mg and oral tab. Cefixime 200 mg bd) 4 months back, and recovered well. Subsequently he presented with the complaints of mild pain,

redness and blurring of vision of the left eye (LE) two months duration. He was diagnosed elsewhere with left eye (LE) intermediate uveitis and investigations revealed reactive lymphocytosis with eosinophilia, increased erythrocyte sedimentation rate (ESR), Toxoplasma IgG, IgM antibodies, Venereal disease research laboratory test (VDRL) and Mantoux test were negative. (Table 1) He was started on oral antibiotics with weekly tapering dose of oral steroids and oral albendazole. His clinical condition did not improve with above medications and he was referred to us for second opinion.

On examination, Best corrected visual acuity (BCVA) in RE 20/20 and in LE 20/32. Intraocular pressure (IOP) was normal in both eyes. Examination of the right eye was normal. Slit lamp biomicroscopic examination of the LE revealed grade 1 nasal pterygium, spheroidal degeneration on the nasal interpalpebral cornea, diffuse pigmented keratic precipitates on the corneal endothelium (Figure 1a) with prominent corneal nerves, grade 1 flare and cells in the anterior chamber, pupil was round, regular and reactive to light and pigments over the anterior lens capsule was noted. Posterior segment examination of the left eye revealed grade 2 vitritis and vitreous haze²⁺, vitreous membranes, mild hyperaemia of the disc and multiple snow ball opacities, and rest of the fundus details were hazily seen (Figure 1b). Corneal sensation was decreased and it was measured by Cochet-Bonnet esthesiometer with the value of 0.5 in the left eye. Spectral Domain Optical coherence tomography (SD-OCT) revealed hyperreflectivity of the inner retinal layer in the midperiphery of the retina (Figure 1d). Fasting blood sugar (FBS), Post prandial blood sugar (PPBS), Glycated hemoglobin (HbA1c) levels were increased (Table 1) with his abnormal CT scan of the chest findings. (Table 2) Confocal microscopy was done to scan the cornea using by the HRT II Rostock Cornea Module (Heidelberg Engineering GmbH, Heidelberg, Germany). In vivo confocal microscopy (IVCM) revealed significant decrease in the sub basal corneal nerve plexus, activated keratocytes in the stroma with highly reflective ovoid shaped cells were present in the in the corneal endothelium suggestive of candida yeast like structure (Figure 1c). Patient underwent anterior chamber paracentesis in the LE and the aqueous humor was analysed for gram stain, Potassium hydroxide (KOH) mount and PCR for panfungal genome, eubacterial genome and SARS CoV-2. Patient received intravitreal 3 voriconazole (100 µg/0.1 ml) injections. Oral steroids were rapidly tapered and stopped. Patient was started on topical and systemic itraconazole therapy. All the investigations came negative and patient did not respond to the medical management. Patient underwent left eye pars plana vitrectomy along with intravitreal voriconazole injection under local anaesthesia. PCR for panfungal genome from vitreous humor sample was positive (Figure 1e) and vitreous humour culture revealed *Candida Tropicalis* confirming the diagnosis of left eye *Candida Tropicalis* endogenous endophthalmitis with post COVID-19 infection. Patient received two more doses of intravitreal voriconazole (50 µg/0.1 ml)

On follow up, after 6 weeks patient visual acuity had improved to 20/25 in the LE. Corneal sensation was normal with the aesthesiometer reading of 1.0. Anterior segment examination revealed flare +, pigments on the anterior lens capsule (Figure 2a) and posterior segment examination was normal following antifungal therapy and pars plana vitrectomy (Figure 2b). Confocal microscopy revealed the presence of dendritic cells with normal nerve fibres (Figure 5c) with normal endothelial cells (Figure 2c). corneal nerve fibre density (CNFD) number of main nerve fibre in mm/mm², Corneal nerve branch density (CTBD)the

total number of branch points per mm², corneal nerve fiber area (CNFA): the total nerve fiber area per mm² values are increased in the post treatment compared to pretreatment scans.(Figure 6)Spectral domain optical coherence tomography (SD-OCT) of the left eye revealed disappearance of the retinal exudates with hyper-reflectivity corresponding to the area of retinal vessel with after shadowing (Figure 2d).

Case 2

A 37 year Asian Indian male, with a recent history of severe COVID-19 infection two months ago, RT PCR for SARS- COV- 2 was positive from the nasopharyngeal swab for which he was admitted for 1 week and he was treated with oral steroids 40mg in tapering doses, T.Favipiravir 1800mg BD first day followed by 800mg BD for next 13 days and T. Ivermectin12 mg OD for 10 days. Ten days later after being diagnosed with COVID-19, he presented with pain, redness and diminution of vision in the LE for which he consulted multiple hospitals and was diagnosed elsewhere with LE panuveitis with hypopyon and investigations done are shown in Table 3. LE anterior chamber paracentesis done elsewhere showed eosinophilic fibrinous material and an occasional lymphocyte. Gram stain smear, potassium hydroxide (KOH) mount and culture for bacteria and fungi were negative. He was started on Dexamethasone + Moxifloxacin eye drops 6 times per day, Homatropine eye drops BD, Nepafenac eye drops BD in the left eye, T. Prednisolone 10 mg OD, T. Itraconazole 100 mg BD, T. Cefixime 200 mg BD, and T. Aceclofenac 100 mg + T. Paracetamol 325 mg BD His clinical condition did not improve with above medications and he was referred to us for second opinion. He was a known case of type II diabetes mellitus on oral hypoglycemic agents (T. Metformin 500mg + T. Glimepiride 2mg). On examination, Best corrected visual acuity (BCVA) in right eye (RE) is 20/20 and in LE is hand motion present. IOP was normal in both eyes. Examination of the RE was normal. Slit lamp biomicroscopy of the left eye revealed hazy cornea with endothelial exudates, grade 3 flare and cells with 1mm hypopyon in the anterior chamber, exudates and membranes covering the pupil, fluffy white clumps on iris was seen (Figure 3a), lens and posterior segment could not be visualized. LE ultrasound B scan revealed increased vitreous echoes of medium reflectivity and retina was attached (Figure 3b). Patient underwent anterior chamber paracentesis in the LE for PCR for panfungal genome which was positive. He also underwent LE diagnostic pars plana vitrectomy with vitreous tap for Grams stain, KOH and culture sensitivity along with intravitreal antibiotics. Vitreous humor analysis revealed no bacterial or fungal growth. Confocal microscopy LE showed multiple hyperreflective ovoid shaped yeast like structures (red arrow)with hyper reflective lines suggestive of hypae /fungal filament(yellow arrow) and well defined cyst (blue arrow), (Figure 3 c). He was started on Prednisolone acetate eye drops 6 times per day, Homatropine eye drops BD, Gatifloxacin eye drops 6 times per day, Itraconazole eye ointment BD in the left eye, T. Ciprofloxacin 500 mg BD and T. Itraconazole 100 mg BD. Since there was increased anterior chamber exudates (ten days after diagnostic vitrectomy), He then underwent LE lensectomy, membranectomy, surgical peripheral iridectomy, core vitrectomy, silicone oil injection with half dose intravitreal injection vancomycin, ceftazidime with voriconazole. Anterior chamber exudative membrane by histopathological examination revealed fungal hyphae within the fibrinous membrane in the anterior chamber of the left eye (Figure 3d) and Grocott

methenamine silver (GMS) stain showing a narrow aseptate non-branching hyphae from the anterior chamber exudate in the left eye confirming the diagnosis of fungal endophthalmitis (Figure 3e & Table 3) Post operatively, his clinical condition improved. On his final visit 3 months later, BCVA in left eye improved to 20/100. Slit lamp biomicroscopy of the LE anterior segment revealed clear cornea with few pigments on the endothelium, grade 1 flare, traces of cells and patent surgical peripheral iridectomy with aphakia (Figure 4a). LE posterior segment showed status post vitrectomy with silicone oil filled eye, disc hyperaemia, flame shaped haemorrhages with attached retina (Figure 4b), with B- scan showed silicone filled eye with attached retina (Figure 4c). Confocal microscopy LE showed multiple hyperreflective resolving ovoid shaped lesions with normal endothelial cells (Figure 4d) Confocal microscopy revealed dendritic cells in the sub basal epithelial area with significant decrease in sub basal corneal nerve plexus (Figure 5a& 5e) along with clusters of highly reflective, irregular shaped cells in the corneal stroma with activated keratocytes (Figure 5 b & 5f) at the time of presentation in both the cases . Follow up scan after 6 weeks in first case and 8 weeks in second case showed increase in immature dendritic cells with improvement in the sub basal corneal nerve plexus (Figure 5c & 5g) with decrease in the numbers of highly reflective irregular shaped cells with the presence of activated keratocytes(Figure 5d & 5h) in the affected eyes . However the cc metric analysis of the nerve fibers revealed decreased corneal nerve fiber length (CNFL): the total length of nerve per mm² and Corneal nerve branch density (CTBD): the total number of branch points per mm². (Figure 6)

Discussion

Ocular manifestations in COVID-19 can present at any stage of the disease. Acute infection causes conjunctivitis, episcleritis and vascular occlusions in retina.² Dysfunctional immune system causes unregulated production of interleukin-6 (IL-6), IL-1 β , IFN- γ , MCP-1, IP-10, IL-4 and IL-10 leading to immune-mediated end-organ damage.⁴ To control the dysfunctional immune system, systemic steroids was used in our cases and tocilizumab was used in the first case. Suppression of pro-inflammatory IL-1 and IL-6 has shown a therapeutic effect in many inflammatory diseases, including viral infections. However, treatment with these interleukin inhibitors, could potentially increase the risk of serious fungal infections, mainly caused by *Pneumocystis jirovecii*, *Histoplasma* spp. and *Candida* spp.⁵ Previous studies have linked IL-6 blockade with an increased risk of opportunistic fungal infection with *Candida albicans*, through inhibition of Th17 lymphocyte proliferation⁶ and our first case is due to *Candida Tropicalis* infection following IL 6 blockade (Tocilizumab) use during COVID 19 infection. In both our patients who were type 2 diabetes we would like to explore the role of concurrent COVID-19 infection. Diabetes is hyper-inflammatory

condition and seems that it may increase susceptibility for COVID-19 independently of other underlying diseases. Postulates include a) impaired immune response (b) hyper- inflammation (b) hyper-coagulable state (c) activation of renin-angiotensin-aldosterone system (RAAS) and dysregulation of sympathetic nervous system d) direct pancreatic islet cell injury. There is evidence that SARS-CoV-2 uses angiotensin-converting enzyme 2 (ACE2) on the surfaces of epithelial cells to bind and enter to infected cells^{7,8}.Both

our patients developed ocular fungal infection post COVID-19. It was possible that patients contracted the infection during the immunosuppressive treatment period which was possibly not detected when they presented earlier with eye symptoms to the local ophthalmologist and the symptoms got worsened following systemic steroid therapy.

In patients with COVID-19 a variety of pathogens, can coexist and cause infection, while the gram negative bacilli and candida are particularly the most common types of bacteria and fungi.^{9,10} Zhang et al¹¹ in a single center study found that in all 221 patients the bacterial coinfection rate is 7.7%, and the fungal co-infection rate is 3.2%.¹¹ It is difficult to distinguish bacterial or fungal infections from existing viral pneumonia based on clinical and radiological performance¹² Calcitonin may also be an auxiliary means for detecting whether there is bacterial or fungal coinfection as the concentration of interleukin (IL)- 1 β , tumor necrosis factor (TNF)- α , and IL-6 increases, which results in the massive production and release of parathyroid derived calcitonin during bacterial infection. However, the synthesis of parathyroid-derived calcitonin is inhibited by (TNF) - γ , which secretion is increased during viral infection¹⁴ In our second patient the procalcitonin levels done at the time of ocular presentation was low negating the possibility of bacterial infection in addition to microbiological test results from the ocular fluids.

In a study of COVID-19 patients, Guo et al¹⁵ investigated the role of diabetes progression and their prognosis. They found that serum levels of inflammation-related biomarkers such as IL-6, C-reactive protein, serum ferritin and coagulation index, and D-dimer were significantly higher in patients with diabetes compared with those without¹⁵ which indicates that diabetic patients are predisposed to an hyper-inflammatory state that ultimately leads to prompt deterioration of COVID-19. This was also seen in both our patients who had higher levels of inflammatory markers.

Following recovery from COVID, when the patients had eye inflammation, it was treated with systemic steroids initially in the first case and antimicrobial therapy and systemic steroids in the second case by the treating physicians and subsequently with no further improvement then the patient was referred to us. Pigmented keratic precipitates, multiple yellowish white fluffy exudates in the vitreous cavity in first case, hypopyon panuveitis with exudates in anterior chamber in the second case suggestive of infectious etiology. Opportunistic infections following post COVID can be due to bacteria¹⁶ viral¹⁷ and fungal infections.³ Among the fungal infections, Mucormycosis following Post COVID 19 infection recently reported from India following systemic immunosuppression. Earlier we have used confocal microscopy to differentiate infectious from non infectious uveitis.¹⁸ Confocal microscopy revealed yeast like structure in both cases and with hyphae and cyst in second case which is similar to earlier reports suggestive of fungal etiology^{19,20,21} Subsequently our observation was confirmed by molecular diagnostic test in both the cases, fungal culture in one case and Histopathological examination (HPE) from the exudates in anterior chamber in the second case. In the first case infection was due to *Candida Tropicalis* and in the second case, we could demonstrate the fungal hyphae both in confocal microscopy and also on HPE examination from the anterior chamber exudates. Presence of budding yeast forms with cyst like structure on confocal microscopy along with presence of fungal hyphae on GMS stain in second case

confirming it is due to fungal infection probably secondary to candidiasis. Avetisov SE et al have reported increased tortuosity of CNF, increase in the number and size of Langerhans cells. In viral uveitis.

In addition to corneal endothelial changes, we also noticed the presence of dendritic cells with decreased in subbasal corneal nerve plexus in both the cases and this observation correlated well with decreased corneal sensation in the first case. We did not test corneal sensation in the second case in view of frank endophthalmitis presentation. Decreased corneal sensation could be explained through corneal nerve involvement. It may be neurotrophic effects of SARS-CoV-2 either directly through viral invasion or indirectly with a mediated immune response^{22,23} with superadded fungal infection.

In our cases, onset of endogenous endophthalmitis was possibly a sequelae of post COVID-19 infection. Correct diagnosis and aggressive therapy with topical, intravitreal voriconazole , systemic itraconazole with PPV and allied procedures resulted in resolution of infection in both the cases. In the first case, clinical presentation was similar to immune mediated uveitis, however confocal microscopy helped us to suspect the infective etiology and inspite of the initial microbiological tests being negative from aqueous humor. Subsequently vitreous biopsy specimen confirmed the presence of Candida tropicalis infection.

In Summary, we report two cases of unilateral endogenous fungal endophthalmitis in post COVID-19 infection in type II diabetic Asian Indian males with confocal findings of the causative organism. Correct diagnosis with appropriate aggressive therapy helped to salvage the vision in this patients. In this report we demonstrated the confocal patterns using IVCM for diagnosis and treatment in endogenous fungal endophthalmitis in post COVID 19 with type 2 diabetes .However future studies are required to explore the use of IVCM in post covid fungal endophthalmitis for the diagnosis and to monitor the therapeutic response to treatment.

Declarations

Ethics committee approval and consent to participate

for this study have been obtained.

Competing interests

The authors declare that they have no competing interests and no funding.

Authors' contributions

PM contributed to the design, acquisition and interpretation of data, and drafted the manuscript. SS contributed to the acquisition of data and edited the manuscript. AS contributed to the acquisition and interpretation of data, and drafted the manuscript. Ak edited the manuscript and in the clinical management.. PG, MK contributed to the acquisition and clinical management. KS contributed to the metric analysis of nerve fibers. RS contributed to the critical revision of the manuscript .All authors read and approved the final manuscript.

Acknowledgement

Dr. Venkata Ramana Anandula, Chief Microbiologist from Narayana Nethralaya, Bangalore India for helping us with molecular diagnostic tests, Mr. Nagaraj, Technician for helping us in confocal microscopy images. Dr. Madhavi Naik MD, Pathologist, St, Theresa Hospital, Rajaji Nagar, Bangalore and Dr. Sanjay A Pai, MD, Consultant, Columbia Asia Referral Hospital, Malleswaram, Bangalore for helping us with H&E and GMS photographs, Dr Deepashri Mutalik, Dr Ramgopal B, Dr. Hardik Nanavati and Dr. K. Bhujang K Shetty, Narayana Nethralaya, Bangalore, India for their support

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Tables

Table -1 Investigational reports of case 1

TEST PARAMETERS	2 months post- COVID 19	4 months post- COVID 19
Complete blood count (CBC)	Lymphocytosis (63%) Eosinophilia (8%)	Normal
Erythrocyte Sedimentation Rate (ESR)	High (42 mm/hr)	12 mm/hr
C- Reactive Protein (CRP)	1.46 mg/L	< 5 mg/L
Serum Urea	28 mg/dl	34 mg/dl
Serum Creatinine	1.05 mg/dl	0.8 mg/dl
Aspartate Amino Transferase (SGOT)	37 U/L	20 U/L
Alanine Amino Transferase (SGPT)	High (82 U/L)	28 U/L
Toxoplasma IgM, IgG	negative	
VDRL	negative	
HIV 1 & 2		negative
HBsAg		negative
Mantoux test	negative	
Fasting blood sugar (FBS)	294 mg/dl	170mg/dl
Post prandial blood sugar (PPBS)	396 mg/dl	224 mg/dl
Glycated hemoglobin (HbA1C)	9.6 %	7.9 %
Urine analysis	Urine sugars++, rest normal	Urine sugars+, rest normal
Urine culture and sensitivity		No growth
Blood culture and sensitivity		No growth
<i>Aqueous aspirate (LE)</i>		
<i>Gram's stain</i>		Amorphous cellular debris, no bacterial agents seen
<i>KOH preparation</i>		No fungal filaments seen
<i>Culture and sensitivity</i>		No growth
<i>PCR for Eubacterial Genome</i>		Negative
		Negative

<i>PCR for Pan fungal Genome</i>	
Lactate dehydrogenase (LDH)	128 IU/L
D- DIMER	112 ng/ml
Ferritin	211.8 ng/ml
TPHA	negative
Serum angiotensin converting enzyme	normal
<i>Vitreous humor biopsy (LE)</i>	
<i>Gram's stain</i>	Occasional inflammatory cells, no organisms seen
<i>PCR for Panfungal genome</i>	Positive
<i>Culture</i>	Candida tropicalis

Table 2 – CT chest findings of case 1

CT CHEST (PLAIN)	
<i>Pre COVID-19 treatment</i>	<i>Post COVID-19 treatment (after 45 days)</i>
Multiple peripheral wedge shaped patchy areas of ground glass opacity in bilateral lungs (=20% lung parenchyma involvement) suggestive of viral pneumonia (COVID-19) - CORADS-5	Diffuse ground glass opacity in bilateral lungs- sequelae of COVID pneumonia with no evidence of active viral pneumonia – CORADS -6
Total CT severity score : 14 /25	

Table 3 – Investigational reports of case 2

TEST PARAMETERS	10 days post COVID	2 months post COVID
Complete Blood Count (CBC)	Leukocytosis (16,330 cells/cumm) Neutrophilia (94.8%) Lymphocytopenia (4.2%) Low eosinophils (0.1%) Low monocytes (0.8%)	Leukocytosis (11,100 cells/cumm) Neutrophils (61%) Lymphocytes (31%) Eosinophils (5%) Monocytes (3%)
C – reactive protein	High (322 mg/L)	Normal
Erythrocyte Sedimentation Rate (ESR)	Normal	Normal
Fasting blood sugar (FBS)	High (260 mg/dL)	High (194 mg/dL)
Post prandial blood sugar (PPBS)	High (362 mg/dL)	High (230 mg/dL)
Glycated hemoglobin (HbA1C)	8.2%	7.4%
Human Immunodeficiency Virus (HIV 1 & 2)	Non reactive	
Hepatitis B surface Antigen (HBsAg)	Non reactive	Non reactive
Hepatitis C Virus antibody (HCV Ab)	Non reactive	Non reactive
B-betacoronavirus and SARS-COV-2 (Nasopharyngeal swab)	POSITIVE	
Mantoux test	Negative	
Serum Urea	High (67.6 mg/dL)	Normal
Serum Creatinine	Normal	Normal
Serum electrolytes	Hyponatremia (129.4 mmol/L)	
Aspartate Amino Transferase (SGOT)	Normal	
Alanine Amino Transferase (SGPT)	High (52.3 U/L)	Normal

Gamma Glutamyl Transferase (GGT)	High (276.1 U/L)	Normal
25- OH Vitamin D (Total)		Normal
Serum Lactate Dehydrogenase (LDH)	Normal	Normal
D- Dimer	High (773.56 ng/mL)	Normal
Serum Procalcitonin		Normal
TEST PARAMETERS	10 days post COVID	2 months post COVID
Serum Ferritin	High (654.26 ng/mL)	Slightly high (329.2 ng/ml)
TPHA		Non reactive
SARS CoV2 receptor-binding domain (RBD) total (IgG/ IgM)		>10 (positive)
Urine analysis	Normal	Normal
Blood culture and sensitivity	No growth	No growth
Urine culture and sensitivity	No growth	No growth
<i>Aqueous aspirate (LE)</i>		
<i>Gram's stain</i>	Eosinophilic fibrinous material and an occasional lymphocyte. No bacterial agents seen	Few pus cells and cellular debris are seen. Bacterial agents could not be made out
	Fungal filaments are not seen	Fungal filaments are not seen
<i>KOH preparation</i>		No growth
	No growth	Negative
		POSITIVE
<i>Culture and sensitivity</i>		Positive fungal filaments
<i>PCR for Eubacterial Genome</i>		

<i>PCR for Pan fungal Genome</i>	
<i>AC membrane for histopathology</i>	
<i>Vitreous humor biopsy (LE)</i>	Occasional pus cells and cellular debris are seen. Bacterial agents could not be made out
<i>Gram's stain</i>	
<i>KOH</i>	Fungal filaments are not seen
<i>Culture and sensitivity</i>	No growth
<i>PCR for Panfungal genome</i>	Negative
<i>Culture</i>	No growth
<i>Novel corona virus 2019 real time</i>	NEGATIVE for B-betacoronavirus and SARS-CoV-2
<i>RT-PCR</i>	

Figures

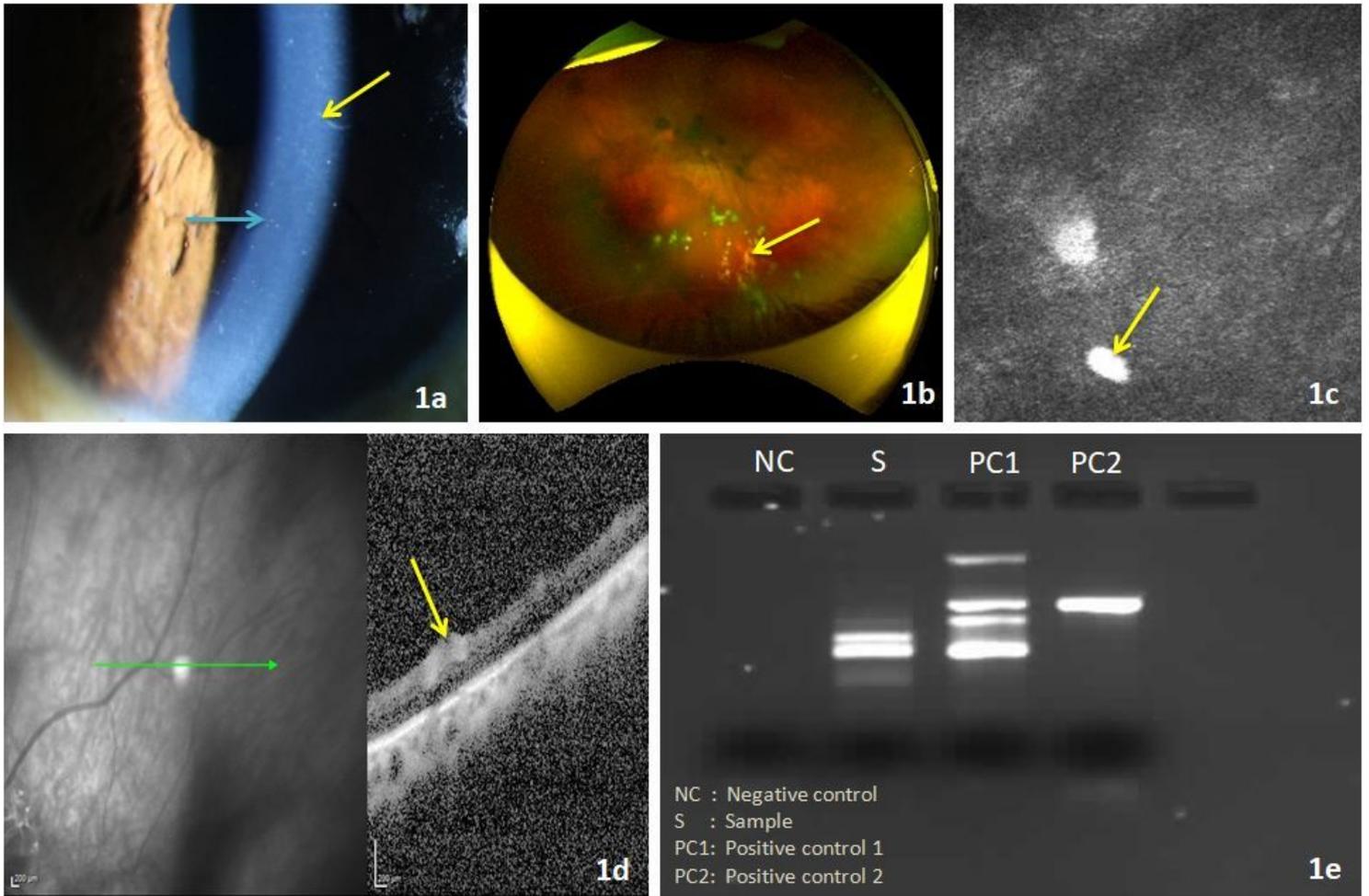


Figure 1

a. Anterior segment photograph of the left eye showing pigmented keratic precipitates and prominent corneal nerves b. OptosTM wide angle fundus photograph of the left eye revealed grade two media haze, multiple whitish opacities with string of beads appearance in the vitreous cavity with attached retina c. Confocal microscopy revealed high fluffy ovoid structures in the corneal endothelium suggestive of candida yeast like structure d. Spectral domain optical coherence tomography (SD-OCT) of the left eye revealed hyperreflective deposit on the inner retinal layer e. PCR 2% agarose gel electrophoresis photograph demonstrating the presence of panfungal genome from the vitreous humor

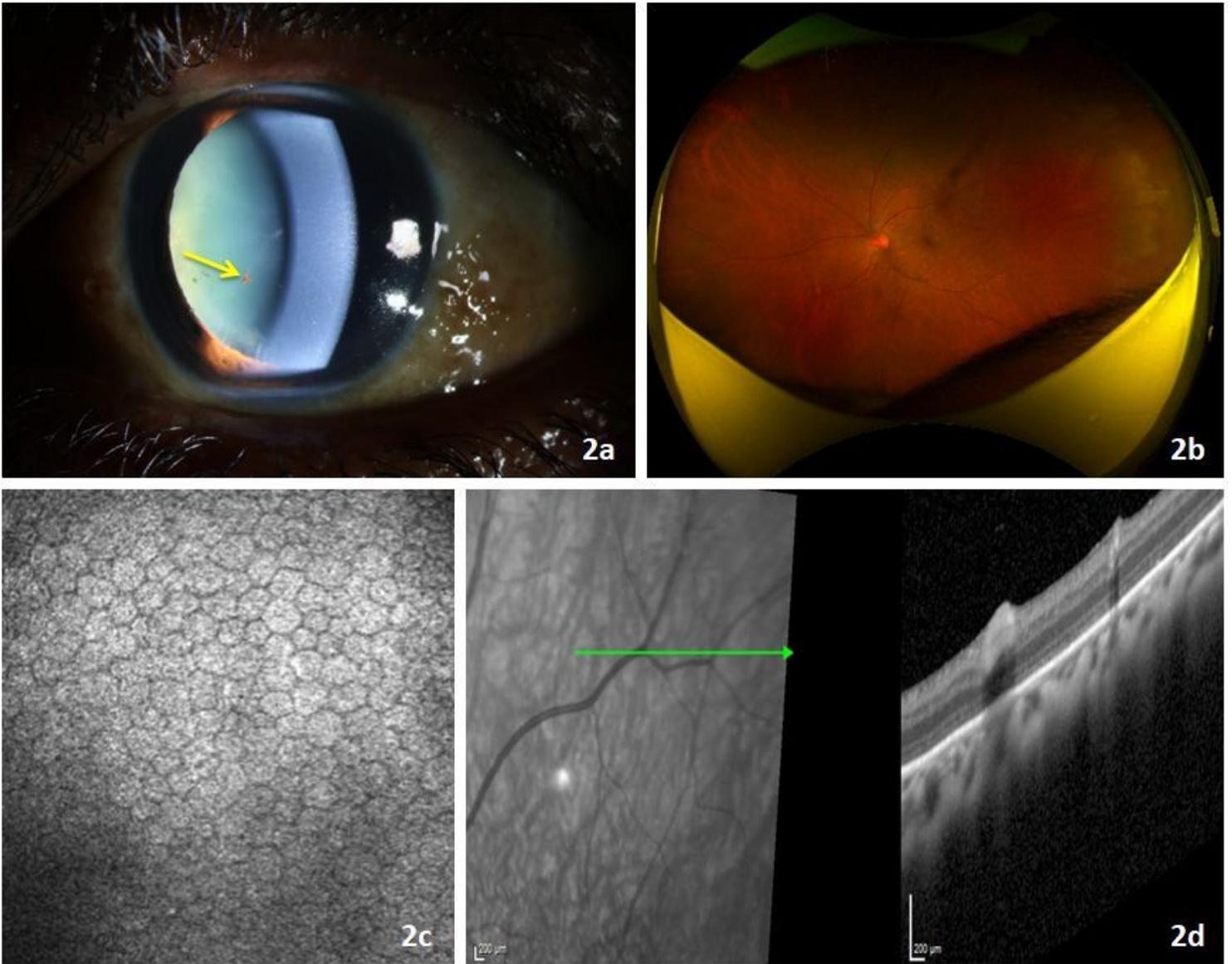


Figure 2

a. Anterior segment photograph of the left eye showing resolved keratic precipitates with pigments on the anterior lens capsule b. OptosTM wide angle fundus photograph of the left eye revealed normal posterior segment in the left eye following antifungal therapy and pars plana vitrectomy c. Confocal microscopy revealed normal endothelial cells with resolution of yeast like structures d. Spectral domain optical coherence tomography (SD-OCT) of the left eye revealed resolution of retinal exudates with retinal vessel shadowing

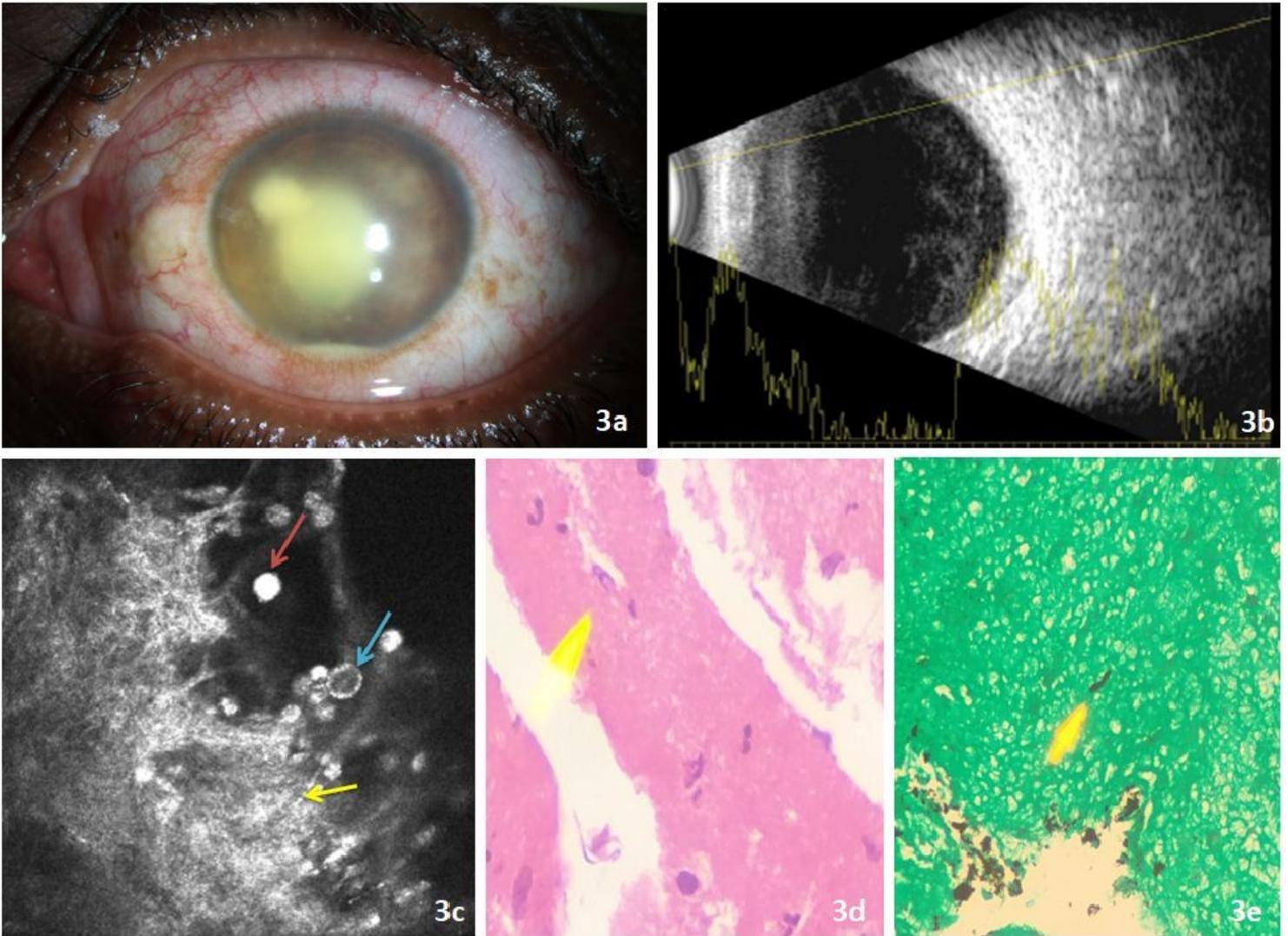


Figure 3

a. Diffuse anterior segment slit lamp biomicroscopic photograph revealed circumferential congestion, exudates in anterior chamber with hypopyon b. B scan ultrasound revealed low to medium reflective echoes in the vitreous cavity suggestive of vitritis with increased retinochoroidal thickness c. Confocal microscopy LE showed multiple hyper-reflective ovoid shaped yeast like structures (red arrow) with hyper-reflective lines suggestive of hyphae /fungal filament(yellow arrow) and well defined cyst (blue arrow) d. Hematoxylin & Eosin stain showing a fungal hyphus (40x, yellow arrow) within the fibrinous membrane in the anterior chamber of the left eye e. Grocott methenamine silver (GMS) stain showing a narrow aseptate non-branching hyphae (40x) (black structure with yellow arrow pointing) from the anterior chamber exudate in the left eye confirming the diagnosis of fungal endophthalmitis

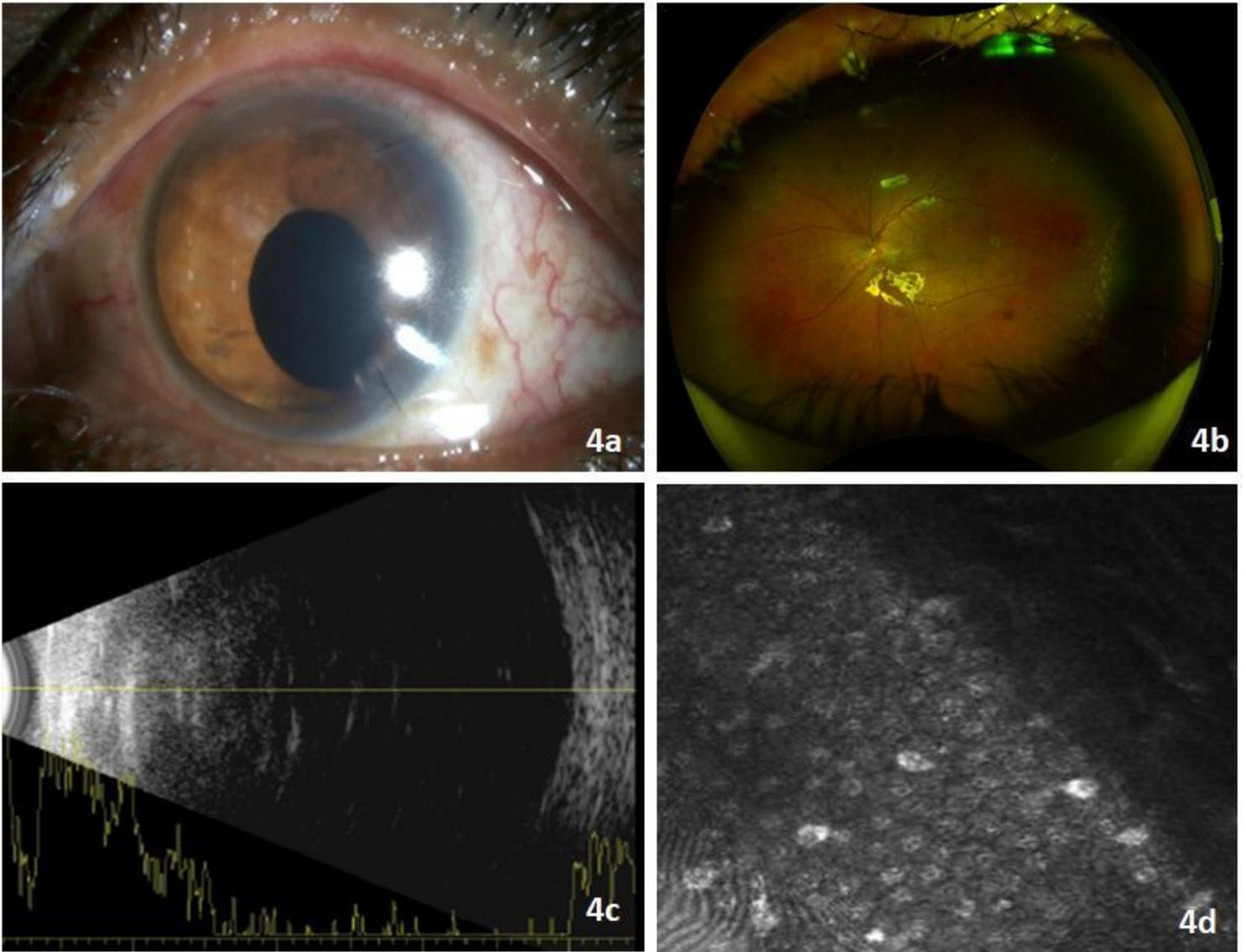
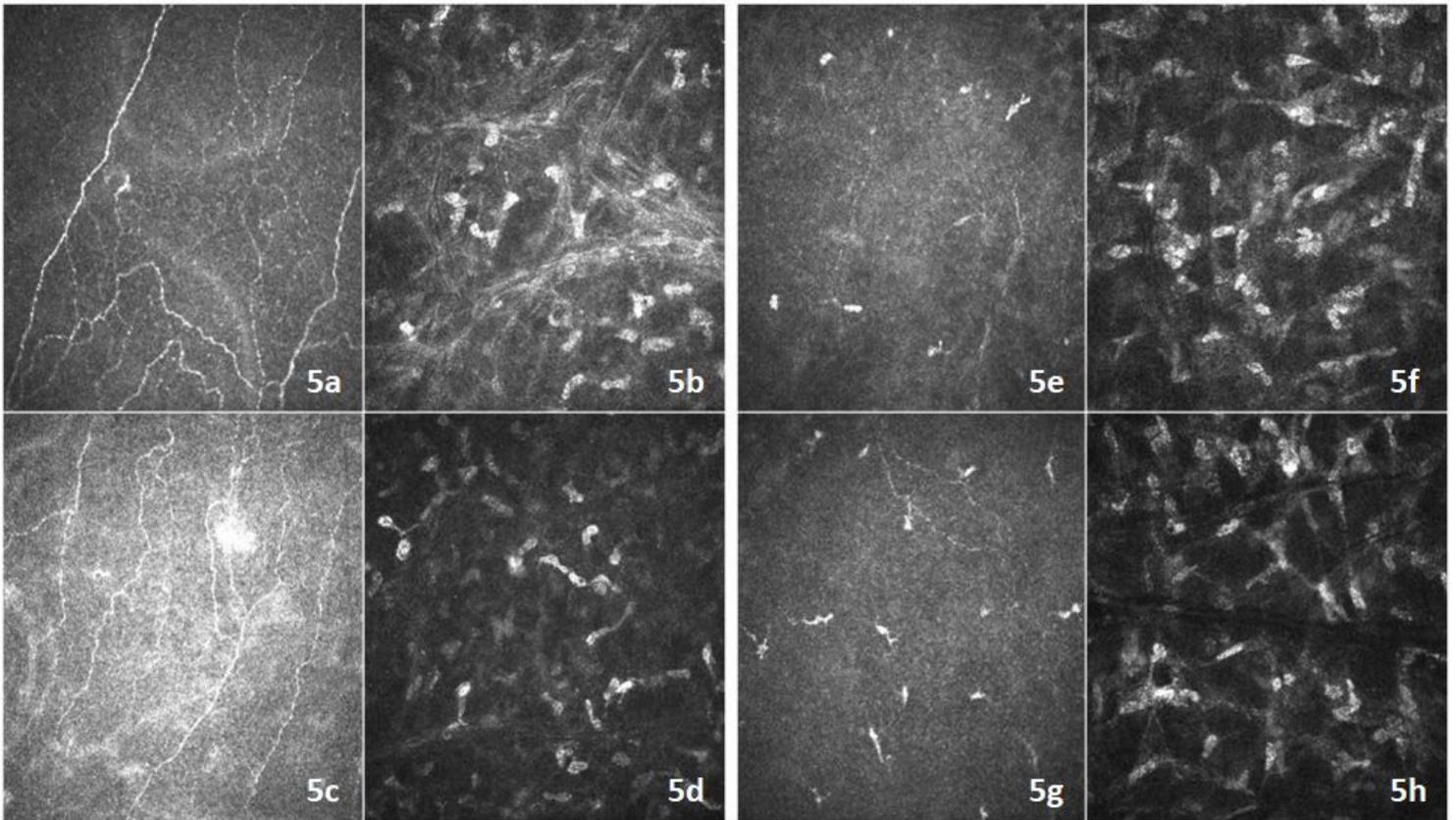


Figure 4

a. Diffuse anterior segment slit lamp biomicroscopic photograph revealed silicone oil in the anterior chamber, patent surgical peripheral iridectomy with aphakia b. Optos™ wide angle fundus photograph of the left eye revealed , disc hyperaemia, flame shaped haemorrhages with attached retina c. B- scan revealed silicone oil filled eye with attached retina d. Confocal microscopy LE showed multiple hyperreflective resolving lesions with normal endothelial cells

CASE 1**CASE 2****Figure 5**

a & e Confocal microscopy revealed dendritic cells in the sub basal epithelial area with significant decrease in sub basal corneal nerve plexus at presentation b & f Clusters of highly reflective, irregular shaped cells in the corneal stroma with activated keratocytes in the both the cases c & g Follow up scan showed increase in immature dendritic cells with improvement in the sub basal corneal nerve plexus d & h Decrease in the numbers of highly reflective irregular shaped cells with keratocytes in the affected eyes

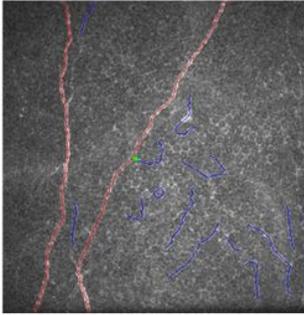
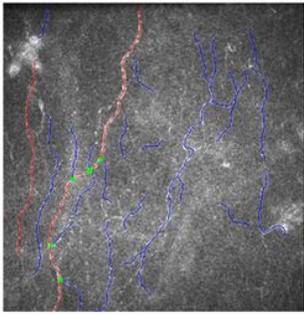
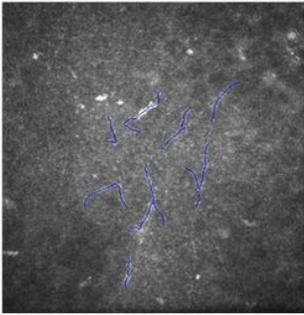
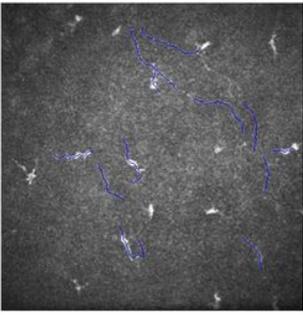
PARAMETERS	CASE 1		CASE 2	
	PRE-TREATMENT	POST-TREATMENT	PRE-TREATMENT	POST-TREATMENT
				
CNFD	14.5824	18.7488	0	0
CNBD	18.7488	16.6656	0	0
CNFL	12.0439	12.0092	4.1058	3.6067
CTBD	29.1648	37.4976	16.6656	8.3328
CNFA	0.0047	0.0058	0.0029	0.0031
CNFW	0.0226	0.0207	0.0299	0.0257
CNFractalDimension	1.4533	1.4438	1.3765	1.3477

Figure 6

The subbasal nerve plexus was quantitatively analysed using automated CC metrics software version 1.0 and the following six parameters were quantified in both the patients. i. Corneal nerve branch density (CNBD): the number of branch points on the main nerve fibers per mm², ii. Corneal nerve fiber length (CNFL): the total length of nerve per mm², iii. Corneal total corneal nerve fiber density (CNFD): the number of nerve fibers per mm², iv. Corneal nerve branch density (CTBD): the total number of branch points per mm², v. Corneal nerve fiber area (CNFA): the total nerve fiber area per mm², vi. Corneal nerve fiber width (CNFW), the average nerve fiber width per mm².