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Detection of Mycobacterium tuberculosis DNA in intraocular fluid of 11 suspected tuberculous uveitis patients by multiplex PCR

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Abstract

Background This study aims to detect Mycobacterium tuberculosis complex (MTBC) DNA in intraocular fluid from clinically suspected tuberculous uveitis patients using multiplex polymerase chain reaction (PCR) and investigate the diagnostic utility of multiplex PCR for tuberculous uveitis.

Methods Primers targeting three specific genes (MPB64, CYP141, and IS6110) within the MTBC genome were designed. Multiplex PCR was conducted using DNA from the H37Rv strain as well as DNA extracted from fluids of confirmed tuberculosis patients to assess primer specificity and method feasibility. Intraocular fluid samples were collected during the initial visit for multiplex PCR detection of MTBC DNA. The results of multiplex PCR tests were correlated with intraocular fluid findings and clinical profiles of patients clinically diagnosed with tuberculous uveitis who underwent standard antituberculosis therapy.

Results Multiplex PCR was employed to detect MTBC DNA in intraocular fluid samples from 15 patients clinically suspected of having tuberculous uveitis, with no amplification bands observed in the DNA lanes for the three target genes. T-cell spot test (T-SPOT) results were positive in 11 patients (100%), while purified protein derivative (PPD) tests were positive in 5 patients (45.5%). Abnormal chest CT findings were noted in 4 patients (36.4%), including one case of active pulmonary tuberculosis and three cases of inactive pulmonary tuberculosis. Retinal vasculitis was observed in 6 eyes (46.2%), panuveitis in 5 eyes (38.5%), and intermediate uveitis in 2 eyes (15.4%). The average duration of antituberculosis therapy administered to the 11 patients was 7.1 months (range: 6–10 months). The medium LogMAR Best Corrected Visual Acuity (BCVA) significantly improved at the last follow-up ($Z=-2.371$, $P=0.018$).

Conclusions Standard antituberculosis therapy demonstrated effectiveness in treating 11 patients clinically suspected of having tuberculous uveitis despite the absence of detectable MTBC DNA in intraocular fluid via multiplex PCR. Further investigation is warranted to elucidate the role of PCR in diagnosing ocular tuberculosis among Chinese individuals.

Keywords Multiplex polymerase chain reaction, Tuberculous uveitis, Mycobacterium tuberculosis complex

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Introduction

It is estimated that nearly one-third of the global population harbors latent tuberculosis (TB) infection, with China ranking third in TB incidence worldwide, following India and Indonesia [1]. Mycobacterium TB complex (MTBC) can infect all ocular tissues except the crystalline lens, with tuberculous uveitis being the most prevalent manifestation [2]. Clinical presentations of tuberculous uveitis are diverse and often mimic other diseases, and obtaining intraocular tissue samples typically requires invasive procedures [3]. Currently, there are no universally accepted diagnostic criteria, leading to a high rate of misdiagnosis. Due to challenges in pathogen culture positivity, diagnosis is frequently empirical, resulting in a significant number of patients being misclassified as having idiopathic uveitis of unknown origin. Inappropriate use of glucocorticoids can lead to severe outcomes such as visual impairment, irreversible blindness, enucleation, or life-threatening conditions [4]. Hence, early and accurate diagnosis is crucial for effective management.

Despite China's status as a major TB burden country, the reported diagnosis rate of tuberculous uveitis remains low. For instance, studies by Lu et al. [5], Zhang et al. [6], and Yang et al. [7] reported relatively low proportions of tuberculous uveitis cases among total uveitis cases (1.54%, 2%, and 0.7%, respectively). In contrast, India has demonstrated a notable increase in tuberculous uveitis diagnosis rates, rising from 0.6% in 1996 [8] to 10.1% in 2004 [9] following the integration of MTBC gene detection via polymerase chain reaction (PCR) into diagnostic criteria. This disparity underscores the potential efficacy of PCR in enhancing diagnostic accuracy for ocular TB. Scholars have advocated for the inclusion of MTBC gene detection results in diagnostic protocols, a practice that has significantly improved diagnosis rates in their setting [10]. However, such approaches are less frequently reported in China, highlighting the need for further research and discourse on optimizing diagnostic strategies for tuberculous uveitis in Chinese populations.

Currently, most reports on PCR detection of MTBC in intraocular fluid with positive results are from India, which contrasts with the epidemiological burden of TB in both India and China [11]. Beyond ethnic and regional disparities, variations in inflammatory involvement, pathogenesis, and detection methods may influence detection rates. In India, approximately 10–40% of MTBC isolates exhibit deletions or have only a single copy of the IS6110 gene, potentially lowering MTBC detection rates when using single-target gene approaches [12]. Recent advancements in multi-target PCR techniques by Indian researchers have substantially increased MTBC detection rates in intraocular fluid. Previous studies indicated sensitivities below 40% for IS6110 and less than 66.6% for MPB64 when used individually [13]. By employing multiplex PCR targeting three specific MTBC genes—IS6110, MPB64, and CYP141—sensitivities have improved to 77.8% with 100% specificity [14]. Notably, studies have shown that the CYP141 gene is present in 97.1% of MTBC isolates, achieving a sensitivity of up to 85.7%, surpassing the sensitivity of other target genes such as protein b (22.2%) [15–17].

In this study, we aimed to detect Mycobacterium TB complex (MTBC) DNA in intraocular fluid from patients clinically suspected of having tuberculous uveitis using multiplex polymerase chain reaction (PCR) and to evaluate the diagnostic utility of this method for tuberculous uveitis.

Materials and methods

Study approval and criteria for patient selection

This study was approved by the Ethics Committee of our institution (Approval number: 2021ky318) and conducted in accordance with the principles of the Helsinki Declaration, and all participants provided informed consent before enrollment. We collected data from 15 patients (18 eyes) with clinically suspected tuberculous uveitis who underwent PCR detection of intraocular fluid at our hospital from December 2019 to December 2021. The inclusion criteria were as follows: (1) Patients meeting diagnostic criteria for tuberculous uveitis [18, 19]: (a) Presenting with uveitis as the primary clinical manifestation, including acute anterior uveitis, chronic granulomatous anterior uveitis, intermediate uveitis, panuveitis, retinal vasculitis, choroidal nodule, multifocal choroiditis, choroidal granuloma, serpiginous choroiditis, among others; (b) Positive T-SPOT or purified protein derivative (PPD) test, presence of active TB focus or calcification focus on chest CT; (c) Exclusion of uveitis caused by other pathogenic factors, such as infectious agents (bacterial, viral, fungal, and parasitic infections), autoimmune disorders, and systemic inflammatory diseases; (2) Received at least six months of effective antituberculosis (anti-TB) therapy; (3) Provided informed consent to participate in the study; (4) Underwent regular follow-up with comprehensive case data recording. The study exclusion criteria were: (1) Presence of severe systemic diseases such as cardiac, cerebral, or renal disorders; (2) Refusal to participate in the study.

Clinical evaluations and diagnostics

All patients underwent comprehensive ophthalmic evaluations, including slit lamp examination, fundus fluorescein angiography (FFA), optical coherence tomography (OCT), and indocyanine green angiography (ICGA). Additionally, chest CT scans and routine serum tests for uveitis-related markers, including infection and immune markers, were performed for all patients. A senior ophthalmologist with more than 10 years in our department

integrated the patients' ocular manifestations, multimodal imaging findings, and laboratory results (including positive T-SPOT or PPD tests and chest CT findings) to exclude other forms of uveitis and clinically diagnose them with tuberculous uveitis.

Sample collection and molecular analysis

Upon obtaining informed consent from patients, basic demographic and clinical data were collected. In aseptic conditions, 100–150 μL of aqueous humor or vitreous humor was collected from each patient, with priority given to the eye displaying more severe inflammation in cases of suspected bilateral tuberculous uveitis. Samples were immediately transferred to the laboratory for DNA extraction and multiplex PCR detection. Collaboration with infectious disease specialists facilitated clinical diagnosis and initiation of empirical anti-TB therapy for one month. In cases where empirical therapy failed to alleviate ocular inflammation or improve visual acuity, treatment was discontinued, and etiological reassessment was conducted. Those who had effective diagnostic anti-TB therapy were follow-up for at least six months after treatment completion, with scheduled visits at 1, 3, 6, and 12 months, ensuring a minimum follow-up period of 12 months. During follow-up visits, visual acuity and ocular inflammation indicators were monitored.

Patients clinically suspected of tuberculous uveitis who responded positively to anti-TB therapy were enrolled in the study cohort. The results of multiplex PCR detection from intraocular fluid samples were analyzed alongside clinical characteristics to further elucidate diagnostic and therapeutic outcomes.

Primer design and validation

The MTBC target gene primers were designed based on sequences retrieved from the NCBI database (http://www.ncbi.nlm.nih.gov/pubmed/) for the coding regions of candidate genes MPB64, CYP141, and IS6110, as well as sequences near the splicing sites of the exon-intron junction regions. The design followed basic principles of primer design using Primer 3.0 online software (http://bioinfo.ut.ee/primer3-0.4.0/), and the synthesized primer s were provided by Bioengineering (Shanghai) Co., Ltd. Details of the designed primers can be found in Table 1.

DNA from the MTBC standard strain H37Rv and DNA extracted from bronchoalveolar lavage fluid, cerebrospinal fluid, and pleural effusion of six clinically confirmed TB patients with positive MTBC culture results were utilized as templates. Primers designed for target genes MPB64, CYP141, and IS6110 were employed in multiplex PCR to amplify target fragments to validate the specificity of the primers and the feasibility of the detection method. PCR conditions included an initial denaturation at 95 °C for 5 min, followed by 33 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min. A volume of 100–150 μL of aqueous humor or vitreous humor was aseptically collected under sterile conditions. All procedures were conducted by the same experienced senior physician. DNA extraction from intraocular fluid was performed using a QIAGEN DNA extraction kit (Germany), following established protocols based on a previous study [14, 20]. The extracted DNA served as the template for multiplex PCR amplification of target fragments, with gel electrophoresis images captured using a Tanon 2500 gel imaging detector.

Data collection and clinical parameters

The clinical data retrieved for the patients with clinically suspected tuberculous uveitis included (1) demographic information such as gender, age, urban or rural residence, history of TB infection or exposure, history of steroid treatment, and main clinical manifestations; (2) Auxiliary eye examinations encompassed (a) Best Corrected Visual Acuity (BCVA) and intraocular pressure: BCVA converted to the logarithm of the minimum angle of resolution (logMAR) using the formula LogMAR = 1 g(1/decimal visual acuity); Visual acuity of hand movements as 3.0, and counting fingers (CF) as 2.0 [21]. (b) slit lamp examination for anterior segment and fundus evaluation; and (c) multimodality imaging modalities such as OCT, FFA, and ICGA; (3) Laboratory assessments comprised routine serum immunology, autoantibody tests, infectivity index-related tests, T-SPOT or PPD test results, and chest CT findings. Patients diagnosed with clinically suspected tuberculous uveitis received one month of diagnostic systemic anti-TB treatment under infectious disease specialist guidance using a combination of isoniazid, rifampicin, pyrazinamide, and ethambutol. In cases of ineffective initial treatment, anti-TB therapy was discontinued, and the etiology reevaluated. Improvement or reduction in ocular inflammation post-treatment prompted continuation of the standard anti-TB regimen, following the WHO-recommended protocol [18]: initial two-month combination therapy with four anti-TB drugs, followed by four months of two-drug therapy (isoniazid and rifampicin). Treatment duration, typically

Table 1 MPB64, CYP141, IS6110 gene amplification primers

Gene	(5′→3′ primer)	(5′→3′ primer)	Amplified fragment size (bp)
MPB64	AAAATTACATCGCCCAGACG	AATCGAAGGCCTTGTACGTG	201
CYP141	CAATGCTGTCGGAACTGAGA	CATTGTCGACGATCTGTTGG	303
IS6110	TTCAGGTCGAGTACGCCTTC	GAACGGCTGATGACCAAACT	323

six months, could be extended to 9–15 months based on treatment response. Oral low-dose prednisone could be administered 1–2 weeks after anti-TB therapy initiation and gradually tapered depending on disease severity and inflammatory response. Treatment decisions were guided by uveitis-trained ophthalmologists, considering disease phenotype, severity, treatment response, and potential impact on vision. Assess the efficacy of anti-tuberculosis therapy using the following criteria: (1) Corrected visual acuity, measured with a standardized logarithmic visual acuity chart and uniformly converted to LogMar units for statistical analysis convenience. (2) Presence of Koeppe nodules, anterior chamber reaction, and quantification of vitreous cellularity [22]. (3) Assessment of the activity level of choroidal inflammatory lesions. (4) Evidence of retinal vascular leakage and macular edema as indicated by FFA or OCT. Should there be an improvement in visual acuity by two or more lines after one month of treatment, or if the vision remains stable with a resolution of inflammatory lesions, the anti-tuberculosis treatment regimen should be continued.

Criteria for response to treatment [18, 19, 23]: Patients were considered to have received effective anti-TB therapy if they underwent treatment for at least six months, experienced improvement or stabilization of visual acuity, subsidence of intraocular inflammation, and remained recurrence-free during the follow-up period. Given the potential hepatotoxicity, neurotoxicity, and retrobulbar optic neuritis associated with commonly used anti-TB drugs, regular monitoring of liver function and general health indicators was conducted throughout treatment [24]. Immediate discontinuation of medication was implemented upon severe drug toxicity or side effects. Patients who responded to diagnostic anti-TB treatment and completed the standard regimen for at least six months underwent follow-up assessments at 1, 3, 6, and 12 months, with a minimum follow-up period of 12 months.

Statistical analysis

All data analyses were conducted using the SPSS software version 25.0. Means and standard deviations are provided for continuous variables, while percentages and proportions are reported for categorical variables. Due to the non-normal distribution of pre- and post-treatment LogMAR BCVA values, the Wilcoxon signed-rank test was applied. A significance level of $P<0.05$ was used to determine statistical significance.

Result

Primer validation results for the target genes MPB64, CYP141, and IS6110

The sizes of the three pairs of target gene segments designed in this study are 201 bp, 303 bp, and 323 bp, respectively. Corresponding amplification bands of these sizes were observed in the agarose gel electrophoresis lanes for both the MTBC standard strain H37Rv and the fluids from TB patients with confirmed TB. No amplification bands appeared in the negative control lane (Fig. 1).

In Fig. 1, L0 denotes the lane with the DNA Marker, which includes six bands ranging from 100 bp to 600 bp, increasing sequentially from bottom to top. Lanes L1-L3 and L4-L6 represent the amplified DNA of MPB64, CYP141, and IS6110 from the Mycobacterium TB standard strain (H37Rv) and DNA extracted from confirmed TB patients, respectively. Amplification bands corresponding to the sizes of the three target genes (MPB64, CYP141, and IS6110) were clearly visible in lanes L1-L3 and L4-L6.

Detection of mycobacterium TB DNA in the intraocular fluid of patients with clinically suspected tuberculous uveitis by multiplex PCR

Intraocular fluid samples, comprising 12 aqueous humor and 3 vitreous humor, were collected from 15 patients. Multiplex PCR analysis revealed no DNA amplification bands corresponding to the three target genes (MPB64,

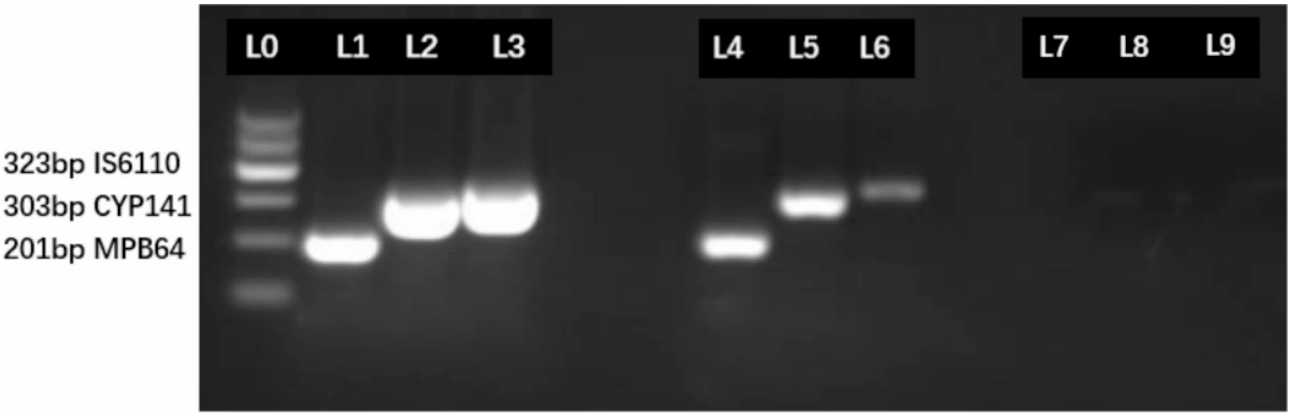

Fig. 1 Results of multiplex PCR for Mycobacterium tuberculosis standard strain H37Rv and DNA from bronchial lavage fluid of a patient diagnosed with pulmonary tuberculosis

CYP141, and IS6110) in any of the intraocular fluid samples (Fig. 2). L0 represents the agarose gel electrophoresis lane containing the Marker, with six bands ranging sequentially from 100 bp to 600 bp. L1-L3, L4-L6, and L7-L9 correspond to lanes containing DNA amplification products from Mycobacterium TB standard strain (H37Rv), intraocular fluid DNA from clinically suspected tuberculous uveitis patients, and negative control samples, respectively. No DNA amplification bands were observed in lanes L4-L6 and L7-L9.

Clinical data for patients with suspected tuberculous uveitis

In this study, 1 patient discontinued treatment due to ineffective diagnostic anti-TB therapy, while 3 patients either discontinued treatment or were lost to follow-up. Among the remaining 11 patients (13 eyes) with suspected tuberculous uveitis, who received standard anti-TB therapy for at least 6 months, treatment was effective. Clinical data for these 11 patients are summarized in Table 2.

Of the 11 cases (13 eyes) treated for clinically suspected tuberculous uveitis, 6 cases (54.5%) were male and 5 cases (45.5%) were female, with an average age of 63.6 years (range: 47–74 years). Three patients (27.3%) resided in urban areas, while 8 (72.7%) lived in rural settings. One patient had a history of tuberculous pleurisy. Four patients (36.4%) had a previous history of steroid therapy. In terms of the main clinical manifestations, 7 cases (63.6%) had iris nodules; 5 cases (45.5%) had vasculitis; 4 cases (36.4%) had choroidal lesions; and no cases developed tuberculoma or Tuberculosis-associated Serpiginous-Like Choroiditis (TB-SLC).

The mean duration of disease at initial presentation was 6.1 months (range: 1–15 months), and the median LogMAR BCVA (Best Corrected Visual Acuity) at initial assessment was 1.3. Following effective anti-TB treatment, among the 11 patients (13 eyes), 6 eyes (46.2%) exhibited simple retinal vasculitis, 5 eyes (38.5%) showed panuveitis and 2 eyes (15.4%) had intermediate uveitis. Additionally, 7 eyes (53.8%) had keratic precipitates, 5 eyes (38.5%) had extensive posterior iris synechiae, 2 eyes (15.4%) had significant snowball opacity in the vitreous, and 2 eyes (15.4%) exhibited macular edema. All 11 patients (100%) tested positive on T-SPOT, with 6 patients (45.5%) also positive on PPD testing. Abnormal chest CT findings were observed in 4 cases (36.4%), including 1 case of active pulmonary TB and 3 cases of fibrous calcification from previous pulmonary TB. The mean follow-up duration for these patients was 13.36 months (range: 12–16 months), during which time inflammation improved without recurrence (Fig. 3). The mean duration of anti-TB therapy administered was 7.1 months (range: 6–10 months).

At the last follow-up, visual acuity improved in 7 patients (8 eyes, 63.4%), while 4 patients (5 eyes, 36.4%) exhibited unchanged visual acuity. Notably, patient 4 experienced improved visual acuity in the left eye, with no significant change in the right eye. The medium LogMAR BCVA at initial diagnosis was 1.3 compared to 0.7 at the last follow-up, indicating a statistically significant improvement in visual acuity post-treatment ($Z=-2.371$, $P=0.018$).

Discussion

Our study found that standard anti-TB therapy effectively treated clinically suspected tuberculous uveitis in 11 patients, despite the absence of detectable MTBC DNA in intraocular fluid via multiplex PCR, highlighting the therapeutic benefit of anti-TB treatment in patients with strong clinical indications of tuberculous uveitis. The paucibacillary nature of intraocular fluid significantly limits the sensitivity of PCR, a challenge well-documented in the literature [25, 26]. Previous studies have shown that the low number of pathogens and limited

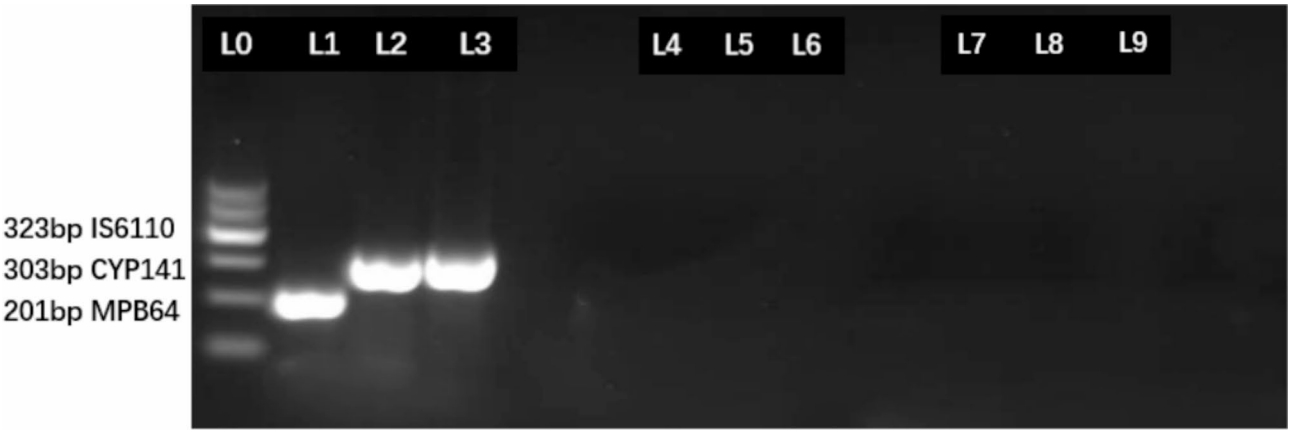

Fig. 2 Results of multiplex PCR detection in the intraocular fluid of patients with clinically suspected tuberculous uveitis

Table 2 Clinical characteristics of 11 patients who responded to antituberculosis therapy

NO.	Age/ Gender	Diagnosis	laterality	Initial BCVA: OD/OS	Last BCVA: OD/OS	Chest CT	T-SPOT	PPD	TB History	ATT (month)	Previous steroid treatment	Iris nodules	Vasculitis	Tuberculoma	Choroidal nodule	TB-SLC	specimen	PCR result
01	58/F	retinal vasculitis	OS	20/20;20/2000	20/20;20/200	-	+	-	-	6	YES	+	+	-	-	-	aqueous	negative
02	61/M	retinal vasculitis	OD	20/400;20/20	20/400;20/20	-	+	+	-	6	NO	-	+	-	-	-	aqueous	negative
03	65/M	retinal vasculitis	OD	20/125;20/20	20/125;20/20	+	+	+	+	8	NO	+	+	-	-	-	aqueous	negative
04	60/M	panuveitis	OU	HM/BE;20/400	HM/BE;20/100	-	+	-	-	10	YES	+	-	-	+	-	aqueous	negative
05	68/M	intermediate uveitis	OD	20/40;NLP	20/25;NLP	+	+	+++	-	6	NO	-	-	-	-	-	aqueous	negative
06	73/F	panuveitis	OS	NLP;20/40	NLP;20/25	-	+	-	-	7	NO	+	-	-	+	-	aqueous	negative
07	46/F	retinal vasculitis	OS	20/20;20/50	20/20;20/25	-	+	++	-	8	NO	-	+	-	-	-	aqueous	negative
08	59/F	panuveitis	OD	20/2000;20/20	20/125;20/20	-	+	+	-	6	YES	+	-	-	+	-	vitreous	negative
09	74/M	retinal vasculitis	OU	FC/20 cm;20/50	20/400;20/25	+	+	++	-	6	NO	+	+	-	-	-	vitreous	negative
10	69/F	intermediate uveitis	OS	20/20;20/200	20/20;20/25	-	+	-	-	9	YES	-	-	-	-	-	aqueous	negative
11	64/M	panuveitis	OD	20/50;20/20	20/50;20/20	+	+	-	-	6	NO	+	-	-	+	-	aqueous	negative

BCVA: best corrected visual acuity; NLP: no light perception; HM/BE: hand movement/before eyes TB: tuberculosis; T-SPOT: T cell spot test; PPD: purified protein derivative; ATT: antituberculosis therapy; Tuberculosis-associated Serpiginous-Like Choroiditis

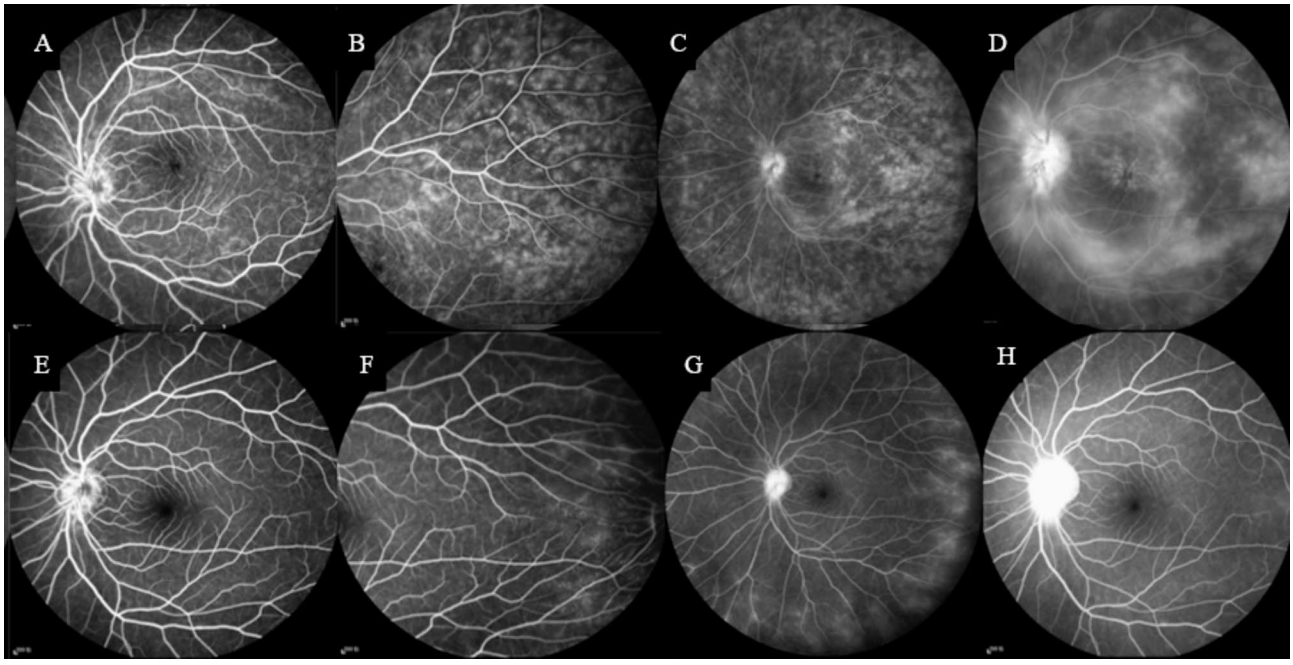

Fig. 3 Fundus fluorescein angiography (FFA) images of a patient with left eye retinal vasculitis before and after antituberculosis therapy. Before treatment (A-D), FFA showed diffuse staining of the retinal vein capillary wall, fluorescein leakage, and late disc strong fluorescence leakage. After standard antituberculosis treatment (E-H), FFA showed no obvious leakage of retinal vessels in the posterior pole and middle periphery, with improved visual acuity

sample volumes often result in negative PCR results despite clinical evidence of infection [27, 28].

As uveitis is associated with infection, obtaining evidence of the pathogen in ocular tissue or intraocular fluid is crucial for diagnosing tuberculous uveitis. However, enhancing the sensitivity of MTBC detection in intraocular fluid remains a challenging issue. Histopathology has confirmed tuberculous uveitis as a paucibacillary infectious disease, characterized by a low number of intraocular pathogens and limited sample volumes, severely restricting the utility of culture and smear techniques [29]. PCR detection methods can overcome these limitations by exponentially amplifying nucleic acids, significantly improving detection sensitivity [30]. The introduction of PCR to diagnose tuberculous uveitis by scholars has notably enhanced detection rates. Kotake et al. [31] pioneered the use of PCR to detect the MPB64 target gene of MTB in the aqueous humor of patients with tuberculous retinal vasculitis, demonstrating its clinical utility in this context.

Indian scholars have reported that multiplex PCR detection can enhance the sensitivity of detecting MTBC in intraocular fluid. Sharma et al. [14] were among the first to utilize multiplex PCR targeting IS6110, MPB64, and protein b genes in patients clinically suspected of tuberculous uveitis, achieving a sensitivity of 77.8% and specificity of 100%. This sensitivity was higher than that of single-gene approaches such as IS6110 or MPB64 aloneand correlated with favorable treatment responses. Praveen et al. [32] conducted multi-target PCR on 114 samples suspected of ocular TB, detecting IS6110 and MPB64 sequences in 80 samples. Only 8 samples tested positive for IS6110 alone. Among the 71 patients who received anti-TB therapy, 91.5% showed positive treatment outcomes. Mohan et al. [33] similarly employed multiplex PCR to assess 13 patients suspected of tuberculous uveitis. Among those with positive MPB64 and protein b sequences, only one patient tested positive for the IS6110 sequence. Considering that 97.1% of MTBC isolates harbor the CYP141 gene, targeting this gene can achieve a sensitivity of 85.7% [15–17], significantly higher than the sensitivity of the protein b gene (22.2%) observed in previous studies. This current study aimed to detect MTBC in intraocular fluid of patients with clinically suspected tuberculous uveitis using multiplex PCR to enhance detection sensitivity. Three primer pairs targeting MPB64, CYP141, and IS6110 were designed based on previous reports from Indian scholars [14, 15, 32]. The specificity of these primers and the feasibility of experimental conditions were validated through repeated testing using DNA from internationally recognized standard TB strains and confirmed TB patients' body fluids.

In this study, we utilized multiplex PCR to detect MTBC DNA in the intraocular fluid of 15 patients (18 eyes) with clinically suspected tuberculous uveitis, encompassing 12 cases of aqueous humor and 3 cases of vitreous body. However, no DNA amplification bands were observed in the lanes targeting the MPB64, CYP141,

and IS6110 genes. Despite negative PCR results, based on clinical manifestations, multimodal imaging, and serological indicators, we maintained a high suspicion of tuberculous uveitis. Accordingly, all 15 patients received diagnostic anti-TB treatment, with those showing effective response subsequently receiving standard anti-TB therapy for at least 6 months. Ultimately, 11 patients (13 eyes) with suspected tuberculous uveitis responded effectively to treatment. In this study, despite effective treatment leading to a clinical diagnosis of tuberculous uveitis in 11 patients, modified multiplex PCR did not detect DNA fragments in their intraocular fluid. This contrasts with previous literature findings, prompting additional analyses. Firstly, we critically reviewed the multiplex PCR methodology employed, selecting IS6110, MPB64, and CYP141 as target genes, consistent with studies by scholars [14, 17, 20]. Secondly, we meticulously designed primers for these targets and rigorously verified their specificity, experimental protocols, and conditions using DNA from the TB standard strain H37Rv, as well as DNA samples from alveolar lavage fluid, cerebrospinal fluid, and pleural effusion of patients clinically diagnosed with pulmonary TB, tuberculous meningitis, and tuberculous pleurisy in our region. All intraocular fluid samples were promptly processed upon collection, utilizing the same QIAGEN DNA extraction kit from the study referenced by Indian scholars [14, 34], with extraction conducted strictly following manufacturer instructions and using consistent sample volumes (100 μL).

We reviewed the literature again and identified limited reports on PCR detection of MTBC in intraocular fluid from patients with tuberculous uveitis in China. Apart from a single case where MTBC DNA was detected by tissue PCR post-ocular evisceration [35], no positive results have been reported in relevant studies using PCR technology to detect MTBC in intraocular fluid [31–34]. Mao et al. in China studied 46 patients with retinal vasculitis and 7 with choroidal tuberculoma who received effective anti-TB treatment. Among these, 5 patients underwent PCR testing for MTBC with no positive results observed [36]. Similarly, Jolly et al. [37] reported that among 19 patients suspected of ocular TB in Hong Kong from 2014 to 2019, 8 patients had negative results in PCR detection of Mycobacterium TB in intraocular fluid. Additionally, other studies noted that despite using RT-PCR, no positive MTBC results were found in two uveitis patients diagnosed with pulmonary nodules [38]. These findings prompted us to analyze potential causes:

The clinical presentation of tuberculous uveitis exhibits variability across different races and regions, leading to diverse clinical phenotypes. These variations suggest differing pathogenic mechanisms for tuberculous uveitis. Currently, two well-recognized pathogenic pathways include direct invasion by MTBC and autoimmune reactions triggered by molecular mimicry [39]. Tabbara et al. [40] proposed that endogenous endophthalmitis, chorioretinitis, and nodular scleritis primarily result from MTBC invasion, whereas anterior uveitis and retinal vasculitis are predominantly immune-mediated. Cyclitis, choroiditis, and multifocal choroiditis may involve elements of both pathways. Rishi et al. [41] demonstrated a high PCR detection rate (up to 100%) for MTBC in cases of active endophthalmitis, contrasting with lower sensitivities observed in non-rapidly progressive, non-suppurative ocular inflammations. Chinese patients more frequently present with retinitis and multifocal choroiditis [37, 42], potentially contributing to the lower MTBC detection rates in intraocular fluid observed in China. In our study, 76.92% of eyes with effective anti-TB treatment exhibited retinal vasculitis, suggesting that immune reactions may be the predominant pathogenic mechanism underlying tuberculous uveitis among Chinese patients. Other factors contributing to the poor detection of Mtb DNA in ocular fluids include the endemicity of TB, anatomical location and load of Mtb, and the integrity of the blood-retinal barrier [11, 43]. Additionally, our study noted that only 46.2% of patients had retinal vasculitis, which may further explain the low detection rates of Mtb DNA in our samples.

Second, in the context of detecting MTBC DNA in Chinese intraocular fluid using PCR techniques, several key considerations warrant further investigation. Firstly, while previous studies, primarily conducted by Indian scholars [14, 15, 32], have identified MPB64, CYP141, and IS6110 as effective target genes with high detection rates, the applicability of these targets in the Chinese population requires validation. Exploration of additional target genes specific to the Chinese demographic could enhance diagnostic sensitivity. Secondly, MTBC possesses a robust cell wall comprising mycolic acid, peptidoglycan, lipoarabinoglycan, and arabinogalactan [44], which provide natural protection against external factors, including DNA extraction methods. Overcoming technical barriers associated with breaking down this cell wall is crucial for improving DNA extraction efficiency. Furthermore, MTBC is a facultative intracellular pathogen residing within host cells, where it may undergo phagocytosis and lysosomal degradation. Consequently, some MTBCs may release DNA into intraocular fluid following interactions with phagocytes and lysosomes. Despite attempts in our study to directly detect MTBC DNA in intraocular fluid without prior extraction, no amplification bands were detected. Future research could explore the specific mechanisms of MTBC interaction with immune cells, particularly retinal pigment epithelial (RPE) cells known for their phagocytic capabilities similar to macrophages [45]. Addressing these challenges and exploring new avenues in PCR detection methodologies could advance our

understanding and diagnostic capabilities for tuberculous uveitis in the Chinese population.

Thirdly, latent infection with MTBC may contribute to alterations in the body's immune status, potentially leading to autoimmune or autoinflammatory diseases, and it has been suggested that latent MTBC infection could trigger abnormal host responses to self-antigens, thereby promoting conditions such as autoimmune uveitis [46]. Yang et al. [47] have investigated the association between TB infection and various forms of non-infectious uveitis. Their large-scale data analysis indicated that a positive T-SPOT status, indicative of latent TB infection, is an independent risk factor for diseases like Behçet's disease. This finding underscores the potential immunological consequences of latent MTBC infection. Additionally, Peng et al. [48] have discussed the association between TB and specific forms of uveitis, such as serpiginous choroiditis. They noted characteristic clinical and fundus imaging features in cases of serpiginous choroiditis with positive PPD (purified protein derivative) and T-SPOT tests, suggesting a link to TB infection.

In the context of diagnosing tuberculous uveitis, multimodal imaging tests play a crucial role alongside clinical presentation, especially when PCR detection of MTBC in intraocular fluid yields negative results. Various findings from multimodal imaging can provide significant clues suggestive of tuberculous uveitis. These include granulomatous anterior uveitis associated with extensive posterior synechiae, retinal vasculitis with or without choroiditis, and serpiginous choroiditis, among others [49]. In our study, despite negative results from multiplex PCR testing for MTBC in intraocular fluid, we diagnosed 15 patients with clinically suspected tuberculous uveitis based on their ocular clinical presentations and comprehensive multimodal imaging examinations. Importantly, we opted to continue anti-TB therapy for these patients, leading to successful treatment completion in 11 patients. This underscores the clinical decision-making process where the absence of positive PCR results does not definitively rule out tuberculous uveitis, especially in the presence of suggestive clinical and imaging findings.

This study had several limitations that should be clarified. First, PCR detection necessitates low nucleic acid concentration in samples, theoretically allowing for testing of aqueous humor, vitreous stock, and diluted vitreous lavage fluid samples [50, 51]. However, due to cost and logistical constraints, primarily aqueous humor samples were used. Future research could consider including additional vitreous humor samples if feasible. Second, the study's sample size was limited. Future studies with larger samples could be conducted to better characterize the clinical features of patients who respond effectively to anti-TB therapy.

Conclusion

The study's findings indicate that despite negative results from multiple PCR tests of intraocular fluid for MTBC DNA, standardized anti-TB therapy can be used to effectively treat clinically suspected tuberculous uveitis. Therefore, clinicians should interpret PCR detection results alongside clinical experience. Further research is needed to understand the pathogenesis of tuberculous uveitis in Chinese patients and to refine PCR detection of MTBC DNA in intraocular fluid.

Abbreviations

MTBC	Mycobacterium tuberculosis complex
PCR	Polymerase chain reaction
T-SPOT	T-cell spot test for tuberculosis infection
PPD	Purified protein derivative test
FFA	Fundus fluorescein angiography
OCT	Optical coherence tomography
ICGA	Indocyanine green angiography
RT-PCR	Reverse transcription PCR
RPE	Retinal pigment epithelial
TB-SLC	Tuberculosis-associated Serpiginous-Like Choroiditis

Supplementary Information

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Supplementary Material 1

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Author contributions

Xu HR was responsible for the acquisition of the clinical information and writing the manuscript. Xu M and Yu J was responsible for explanations of all the results and reviewing the manuscript. Chen H, Du W and Chen F were responsible for reviewing the manuscript. All authors read and approved the final manuscript.

Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Subei People's Hospital affiliated to Yangzhou University (Approval number: 2021ky318) and conducted in accordance with the provisions of the Helsinki Declaration. All participants signed an informed consent form before enrolling in the study.

Consent of publication

Not applicable.

Competing interests

The authors declare no competing interests.

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