Clinical and laboratory features of PCR-confirmed periocular tuberculosis in China

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INTRODUCTION

Periocular tuberculosis (TB) refers to mycobacterium tuberculosis (MTB) infection around the eye, and on its surface[1]. It comprises many subtypes, including TB of eyelid, conjunctiva, cornea, sclera, nasolacrimal system and other intraorbital tissues. Although the prevalence is low, periocular TB can cause devastating outcomes[2]. Therefore, experts need to fully investigate the clinical and laboratory features of periocular TB. MTB culture sensitivity in extrapulmonary TB is very low, fluctuating from 11.9% to 34.2% in different studies[3-4]. A substantial amount of periocular TB cases was diagnosed presumptively without pathogenic evidences, and these cases may mislead understanding of periocular TB clinical features[5]. Recently, nested polymerase chain reaction (nPCR) shows advantages in extrapulmonary TB diagnosis[6]. The technique amplifies one gene target with two pairs of primers in two rounds of amplification, resulting in more abundant products and lower mispairing rate[7]. More and more studies adopt nPCR as gold standard to diagnose tuberculous infection. We reviewed medical records of presumptive TB cases in our hospital, performed nPCR test to obtain definitive diagnosis, and carefully analyzed the clinical and laboratory features of polymerase chain reaction (PCR)-confirmed periocular TB.

METHODS

Briefly, we reviewed all medical records of periocular TB in adults (older than 18 years old) without human immunodeficiency virus (HIV) from 2007 to 2017. The study protocol was in accordance with Declaration of Helsinki (2008), and approved by the Institutional Review Board. Informed consent was obtained from all patients. Inclusive criteria were suggestive clinical symptoms/signs and positive response to anti-tuberculous therapy (ATT). The criteria are consistent with WHO diagnostic criteria of extrapulmonary TB[8]. Cases with follow-up less than two years were excluded. To confirm diagnosis, we performed nPCR test targeting IS6110 with formalin-fixed paraffin-embedded specimens as Ritis et al[8] reported. Clinical features of confirmed periocular TB cases were then summarized, including demographics, medical history, ophthalmic examination, systemic evaluation, and various imaging results.

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AFB staining was performed with Ziehl-Neelsen method in at least ten slides from each paraffin block. Other laboratory tests were reviewed, including blood routine test, tuberculin skin test (TST) and interferon γ release assay (T-SPOT). Correlation analysis was conducted between different variables and three important TB parameters, including caseous necrosis, positive AFB staining and positive T-SPOT result. ATT therapy and follow-up results were summarized, and data analysis was conducted between different variables and three important TB parameters, including caseous necrosis, positive AFB staining and positive T-SPOT result. ATT therapy and follow-up results were summarized, and data analysis was carried out to examine the influential factors for ATT treatment results. $P$ value less than 0.05 is statistically significant.

**RESULTS AND DISCUSSION**

**Clinical Features** Twelve cases complied with the inclusive criteria of presumptive periocular TB. Nine cases (10 eyes) were confirmed by nPCR test, including one lupus vulgaris of eyelid, two tuberculosis conjunctivitis, one tuberculosis anterior scleritis, and five orbital TB (Table 1). Age range was 23 to 78 years old (mean 59.75, median 63). Male to female ratio was 4 to 5. All lesions were unilateral except for a unilateral tuberculosis dacryoadenitis (case 9). One patient complained of vision loss and ocular pain due to tuberculosis anterior scleritis (case 4). Lupus vulgaris (case 1) presented with chronic swelling and painless ulceration of eyelid (Figure 1A). Conjunctival TB manifested as either nodular (Figure 1B) or diffused (Figure 1C) hyperplasia with purulent discharge. Orbital TB was insidious, and presented with cold, nontender soft tissue swelling. On CT scan, the lesions generally presented as medium density mass with moderate enhancement, and sometimes presented with bony erosion and abscess formation (Figure 1D, 1E). On MRI scan, the lesion signals were commonly iso-/hypointense on T1 images (Figure 1F), iso-/hyointense on T2 images, and heterogeneous on enhanced images (Figure 1G). Other symptoms included progressive proptosis, eye position deviation and ocular movement defects. At the time of presentation, two patients suffered systemic symptoms such as fatigue, weight loss and poor appetite. No patients had chest symptoms including breathless, hemoptysis, or chest pain. All patients were immunocompetent, and five out of nine had diabetes. Chest X-ray was conducted in eight cases, with no active pulmonary lesions discovered. One case underwent chest CT scan, and was detected with fibrotic strips at right lung apex. Regarding to TB related medical histories, all patients had bacille Calmette-Guerin vaccination after birth. Two patients recalled TB contact history during childhood. One patient had a history of tuberculous lymphadenitis.

**Laboratory Features** T-SPOT test was positive in six out of seven patients (85.7% sensitivity). Four patients were examined with TST test, and all showed positive reaction with induration larger than 10 mm. On HE staining, all cases showed granulomatous inflammation, and three of them presented caseous necrosis (33.3% sensitivity). On AFB staining, four out of nine cases were confirmed with positive results (44.4% sensitivity). Based on blood tests, lymphocyte percentage (in all blood cells) decreased in four out of nine cases (44.4%), erythrocyte sedimentation rate accelerated in two out of three cases (66.7%), C-reactive protein elevated in one out of three cases (33.3%), and mild anemia was discovered in two out of nine cases (22.2%). Other blood tests including angiotensin converting enzyme, auto-antibodies, hepatitis and syphilis infection showed nothing special. CT or MRI scan was performed in orbital TB patients.

**Treatment and Follow-up** All patients were prescribed with two-stage ATT: four drugs (isoniazid, rifampin, pyrazinamide, ethambutol) for the first two months, and two drugs (isoniazid, rifampin) for the next five to seven months. The average treatment duration was 8mo. Five patients suffered delayed...
diagnosis as a result of underdiagnosis or misdiagnosis. These patients had longer ATT therapy (one tailed $t$ test, $P=0.031$) than timely diagnosis patients, indicating that delayed diagnosis may be a risk factor for longer ATT duration. After withdrawal of ATT therapy, seven patients recovered completely without vision loss. One patient had slight vision loss due to optic nerve compression (visual acuity from 0.8 to 0.6, International Standard Chart). One patient suffered complete vision loss due to enucleation (refer to case report section).

**Data Analysis** Correlation analysis was conducted between important tuberculous parameters (T-SPOT, AFB staining, caseous necrosis and ATT duration) and potential influence variables (Table 2). Based on data analysis, low lymphocyte percentage (Spearman’s Rank, $P=0.019$) and high monocyte-lymphocyte (M/L) ratio (Spearman’s Rank, $P=0.042$) positively correlate with positive AFB staining. Male gender (Fisher exact probability, $P=0.048$) and Langhans giant cell (Fisher exact probability, $P=0.048$) positively correlate with existence of caseous necrosis. AFB staining and caseous necrosis positively correlate with each other (Fisher exact probability, $P=0.048$). No variables showed correlation with T-SPOT test and ATT duration. The results suggested that male patients with decreased lymphocytes, as well as Langhans giant cells on HE staining, were more prone to be diagnosed with periocular TB.

**Case Report** A 56-year-old female farmer came to our hospital. Her chief complaint was blurred vision, recurrent redness, and ocular pain for ten months. Slit-lamp examination showed scleral nodules and anterior chamber inflammation. After ruled out autoimmune diseases, we performed TST and T-SPOT tests. Both tests showed positive results, and highly suggested tuberculous infection. To confirm diagnosis, we conducted scleral biopsy. HE staining showed granulomatous inflammation and caseous necrosis (Figure 2A); fungal staining and AFB staining were negative; nPCR test was positive (Figure 2B). We made the diagnosis of anterior tuberculous scleritis, and subscribed ATT treatment for nine months. Six months later, she came back to our hospital, with complete vision loss, intractable pain and large amounts of purulent discharge. She recalled that her eyes were scratched by plants a month ago. When asked about ATT treatment, she arbitrarily stopped taking drugs three months ago because of symptom relief. On physical examination, the right globe was fixed, with hypopyon and tenderness. B-scan showed complete
retinal detachment, thinning sclera and surrounding soft tissue edema. The patient was diagnosed with panophthalmitis, and underwent enucleation. Histopathological analysis showed untypical granulomatous inflammation (Figure 2C), with positive fungal staining (Figure 2D), positive AFB staining (Figure 2E) and positive nPCR result. The final diagnosis was fungal-tuberculous coinfection. Insufficient treatment may be the cause of double infection. We restarted both anti-tuberculous and anti-fungal therapy, and the patient recovered completely after nine months.

Nested PCR technique is a major breakthrough in diagnosis of extrapulmonary TB. The novel technique shows better performance than traditional PCR and other ancillary TB tests, especially in paucibacillary extrapulmonary specimens[9]. Many in-house nPCR tests are available based on amplified targets. The most widely used target is IS6110, a multi-copy sequence randomly distributing throughout MTB genome[10]. Diagnostic efficacy of nPCR is still unsure in periocular TB, and we suggest further study to explore the performance of nPCR technique in diagnosis of periocular TB. Based on our study, male gender, decreased lymphocyte count and high M/L ratio are three important parameters to assist TB diagnosis. Similar gender bias was previously reported in other TB studies[11-12], and the gender pattern is probably reversed in Caucasian studies[13-14]. M/L ratio was adopted as TB biomarker in many previous TB studies[15], and was used to monitor ATT response.

<table>
<thead>
<tr>
<th>Variables</th>
<th>T-SPOT</th>
<th>AFB staining</th>
<th>Caseous necrosis</th>
<th>ATT duration</th>
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<tbody>
<tr>
<td>Continuous variables</td>
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<td>Age</td>
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<td>Symptom duration(^b)</td>
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<td>Lymphocyte percentage(^c)</td>
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<td>Monocyte-lymphocyte ratio</td>
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<td>Neutrophil-lymphocyte ratio</td>
<td>0.182</td>
<td>0.076</td>
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<tr>
<td>Dichotomous variables</td>
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<tr>
<td>Sex</td>
<td>0.571</td>
<td>0.167</td>
<td>0.048*</td>
<td>0.500</td>
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<tr>
<td>Site of lesion(^d)</td>
<td>0.714</td>
<td>0.317</td>
<td>0.595</td>
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<td>Shape of lesion(^e)</td>
<td>0.143</td>
<td>0.167</td>
<td>0.583</td>
<td>0.109</td>
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<tr>
<td>Intervention(^f)</td>
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<td>0.167</td>
<td>0.583</td>
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<td>0.643</td>
<td>0.405</td>
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<td>Langhans giant cell</td>
<td>0.571</td>
<td>0.167</td>
<td>0.048*</td>
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<td>0.714</td>
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<tr>
<td>AFB staining</td>
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<td>-</td>
<td>0.048*</td>
<td>0.156</td>
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<tr>
<td>Caseous necrosis</td>
<td>0.714</td>
<td>0.048*</td>
<td>-</td>
<td>0.500</td>
</tr>
</tbody>
</table>

AFB: Acid-fast bacilli; T-SPOT: Interferon γ release assay; ATT: Anti-tuberculous therapy. *Significant difference. \(^b\)Refers to symptom duration from onset to final diagnosis; \(^c\)Lymphocyte percentage in all blood cells; \(^d\)Divided into ocular surface lesions (including lacrimal gland) and orbital lesions; \(^e\)Divided into nodular lesions and diffuse lesions; \(^f\)Divided into biopsy group and surgical removal group.
during treatment of pediatric osteoarticular TB\cite{16}. Coinfection is an important issue in TB patients. According to WHO report in 2017, TB-HIV coinfection has drawn worldwide attention\cite{17}. However, few studies focus on TB coinfection in immunocompetent patients. Therefore, coinfection is an unneglectable issue in both immunosuppressive and immunocompetent patients. To conclude, our study helps health workers to grasp more profound comprehension of clinical and laboratory features of periocular TB, and offers insights into the essential role of nested PCR technique for periocular TB diagnosis.

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**REFERENCES**