Role of Immuno-Polymerase Chain Reaction (I-PCR) in Resolving Diagnostic Dilemma Between Tuberculoma and Neurocysticercosis: A Case Report

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Patient: Female, 17
Final Diagnosis: Tuberculous meningitis
Symptoms: Headache • vomiting • loss of appetite
Medication: —
Clinical Procedure: —
Specialty: Infectious Diseases

Objective: Unusual clinical course
Background: Tuberculoma and neurocysticercosis (NCC) often show similar clinical and neuroimaging features. Differential diagnosis of these 2 diseases is imperative, as tuberculoma is an active infection that requires immediate antitubercular therapy (ATT).

Case Report: We present the case of a 17-year-old Indian girl with fever, severe headache, and right 6th cranial nerve palsy. Brain magnetic resonance imaging (MRI) showed multiple tiny ring-enhancing lesions in bilateral cerebral parenchyma with mild perilesional edema, which were initially thought to be NCC, but subsequently were diagnosed as brain tuberculomas. Based on clinical findings, mildly increased choline/creatine ratio (1.35) with slight prominent lipid lactate peak and absence of alanine, succinate peak by magnetic resonance spectroscopy (MRS), and the detection of Mycobacterium tuberculosis (Mtbc)-specific early-secreted antigenic target-6 (ESAT-6, Rv3875) protein from the cerebrospinal fluid (CSF) by indirect ELISA, as well as indirect immuno-PCR (I-PCR) assay, diagnosis of brain tuberculomas associated with tuberculous meningitis (TBM) was confirmed, which was followed by ATT. The patient responded well and the symptoms resolved.

Conclusions: In this case, multiple ring-enhancing lesions of the brain by MRI were diagnosed as tuberculomas associated with TBM by MRS and indirect ELISA/I-PCR method, thus resolving the diagnostic dilemma.

MeSH Keywords: Immuno-Polymerase Chain Reaction • Neurocysticercosis • Tuberculoma
Background

Neurotuberculosis accounts for 2–5% of all tuberculosis (TB) cases and up to 15% of AIDS-related TB. It causes high morbidity and mortality, predominantly in children of endemic countries, including India [1]. Tuberculomas are unique features of central nervous system (CNS) TB that reveal clinical findings similar to NCC caused by Taenia solium [1,2]. Hematogenous spread of tubercle bacilli from the lungs is most common, leading to small subpial infective foci known as rich foci, which form a reservoir for the appearance of intracranial manifestation [1,3,4]. However, tuberculomas also appear with symptoms and signs of focal neurological deficits without systemic disease [1,3]. The conventional neuroimaging modalities such as computed tomography (CT) scan and MRI cannot differentiate the multiple ring-enhancing lesions associated with diseases such as sarcoidosis, neurosyphilis, malignant lesions, pyogenic abscess, cryptococcosis, and toxoplasmosis, as well as NCC and tuberculomas [2,5,6]. MRS is considered a relatively reliable modality to resolve the diagnostic dilemma between NCC and tuberculomas, as increased lipid peak, >1.0 ratio of choline/creatinine, absence of alanine, succinate peak, and decreased level of N-acetylaspartate (NAA) have been demonstrated in tuberculomas formation [6,7]. The utility of GeneXpert (MTB/RIF) assay for the detection of tuberculomas has also been documented [1]. I-PCR, an ultrasensitive method, combines the simplicity and versatility of ELISA with the enormous amplification capacity of PCR, thus leading to a several-fold increase in sensitivity in comparison to analogous ELISA [8,9]. We previously demonstrated the utility of I-PCR based on the detection of potential Mtb antigens for rapid and accurate diagnosis of pulmonary and extrapulmonary TB, including TB pleuritis, TB lymphadenitis, and abdominal TB [10–12], but no report is available on the differential diagnosis of cerebral tuberculomas with I-PCR. In the present case, diagnosis of tuberculomas associated with TBM was made by MRS report and the detection of Mtb ESAT-6 protein in CSF by indirect ELISA/I-PCR.

Case Report

A 17-year-old Indian girl was referred to the Neurology Department, UHS, Rohtak, in March 2017 with history of holocranial headache, vomiting, and loss of appetite for the last 15 days. Her past medical history was negative for neurological/medical conditions. She was conscious, oriented, and afebrile, with blood pressure of 100/60 mm of Hg and pulse rate of 68/min. On systemic examination, cardiovascular, respiratory, and gastrointestinal systems were normal. Her detailed neurological evaluation revealed normal Mini-Mental Status Examination (MMSE) of 30/30. All cranial nerves were normal except for bilateral (b/l) papillodema. Motor and sensory examination was normal. Her blood biochemistry and hematological examination were normal and ESR was 41 mm in the first hour. The viral markers (hepatitis B, hepatitis C, and HIV) were negative. No extracranial tubercular lesions were found. Her chest X-ray and whole-abdomen ultrasound results were normal. MRI of the brain revealed multiple nodular and ring configuration lesions in b/l cerebral parenchyma and left ganglio-capsular region, pons, and medulla, with mild perilesional edema attributed to probable NCC (Figure 1A, 1B). Lumbar puncture (LP) was performed and CSF analysis showed 35 mg/dL protein, 55 mg/dL sugar with corresponding blood sugar of 94 mg/dL, TLC 5 cells/mm³ (90% lymphocytes, 10% polymorphonuclear cells), and adenosine deaminase level was ~2.29 U/L. Indian ink/cryptococcal antigen, Mantoux test, smear, and culture examination of CSF for pyogenic bacteria and Mtb were all negative. Based on these clinical observations, the patient was suspected to have NCC and was treated with anti-emic and anti-edema measures (Decadron and acetazolamide) but was not given any cysticidal drugs. After 15 days of treatment, no focal neurological deficits were observed. Except for mild headache, other symptoms such as vomiting and anorexia subsided. The patient was fully conscious, alert, and performing all the routine activities independently and was discharged in stable condition.

Two months later, she was again admitted to our hospital due to headache, vomiting, and restlessness, although she was afebrile with no focal neurological deficits. During her stay in the hospital for 1 week, she became drowsy, developed right 6th cranial nerve palsy and was febrile. However, b/l papilledema was persisting. Repeat brain MRI demonstrated multiple tiny ring configurations as described in Figure 1A, 1B. LP was repeated and showed normal CSF pressure, TLC- 40 cells/mm³, low glucose (44 mg/dL), and elevated protein (134 mg/dL), which suggested chronic meningitis with possibility of TBM. Because of persistent altered consciousness, the brain CT scan did not show hydrocephalus or any additional lesions. MRS showed a moderately enhanced choline/creatinine ratio of 1.35 with slight lipid lactate peak, absence of alanine, succinate peak, and mildly decreased NAA peak (Figure 2); these findings revealed the probability of tuberculomas rather than NCC, but the diagnosis remained unresolved. CSF was evaluated for acid-fast bacilli (AFB) smear, culture, multiplex PCR (M-PCR targeting IS6110 and mpb64), ELISA, and I-PCR tests. Smear, culture, and M-PCR were negative; however, the baseline level of Mtb ESAT-6 (7.56±0.05 ng/mL, done in triplicate) was detected by indirect ELISA, which was confirmed by indirect I-PCR method using a streptavidin-biotin system [10]. I-PCR revealed a clearly visible band of ESAT-6 on 1.5% agarose gel electrophoresis. On the basis of MRS, ELISA, and I-PCR results, TBM with multiple tuberculomas was strongly suspected. Five-drugs ATT with steroids and antiepileptics were initiated. Interestingly, the patient responded well and became fully conscious without any focal neurologic deficit during the next 2–3 weeks, thus
indicating that she had tuberculomas. A brain MRI repeated at ~2 months also clearly revealed a significant decrease in number and size of small nodular lesions and the resolution of ring configuration lesions with decreased perilesional edema in b/l cerebral parenchyma, left ganglio-capsular region, pons, and medullas (Figure 1C, 1D).

**Discussion**

Diagnosis of CNS tuberculomas is elusive, compelling a high index of suspicion due to its similarity to NCC. Differential diagnosis of these 2 diseases is crucial, as NCC is relatively benign and self-limiting, whereas CNS tuberculomas is a highly active infection requiring timely ATT [6]. Tuberculomas and NCC lesions resemble each other in many aspects on contrast-enhanced CT and contrast MRI, but the differentiation of these 2 granulomas can be made on the basis of location, number of lesions, various stages, enhancement pattern, and the constitutional symptoms [6,13]. MRS usually displays high lipid peaks in tuberculomas, while amino acid peaks are seen in NCC [4,6]. Moreover, tuberculomas are generally solitary, but multiple nodular ring-like enhancing lesions similar to NCC are also found in 15–34% of CNS TB, which often confuse the diagnosis [4,14].
In this case, initial brain MRI showed multiple lesions with characteristics similar to different stages of NCC, whereas MRS revealed the possibility of tuberculomas. Strikingly, ESAT-6 was detected in CSF by ELISA, which was further confirmed by I-PCR assay. Similarly, early diagnosis of TBM cases has been demonstrated based on the detection of ESAT-6 in CSF samples by ELISA [15]. In fact, ESAT-6 is a highly Mtb-specific protein found in asymptomatic patients and individuals who are at risk of activation of latent TB [16,17]. ESAT-6 is also considered as an immunodominant T cell-stimulatory antigen that is recognized by specific interferon gamma (IFN-γ) secreting T cells, which are present in greater numbers in TB patients who have active infection in comparison to those of uninfected individuals [16], but detection of IFN-γ in blood cannot differentiate between active and latent TB [17]. Our patient presumably had latent TB that was reactivated, thus leading to brain tuberculomas associated with TBM [14]. However, it is strongly suspected that the CNS manifestation arise as disseminated TB from the lungs [1,4].

I-PCR has been documented to be a rapid, robust, and highly sensitive method for the detection of mycobacterial antigens up to picogram levels from sputum and pleural fluids of TB patients [9,18]. We demonstrated the utility of I-PCR for the diagnosis of pulmonary and extrapulmonary TB, including paucibacillary smear-negative suspected pleural TB patients with good sensitivities (72–83% for pulmonary TB and 62–77% for pleural TB) and specificities (85–93%) based on the detection of array of Mtb antigens, i.e., regions of differences encoded secreted proteins such as ESAT-6 and MPT-64 (Rv1980c), as well as Ag85B (Rv1886c), PstS1 (Rv0934), and cord factor (trehalose 6,6’-dimycolate) in body fluids, which revealed its superiority over ELISA [9–11,18]. We also found that I-PCR based on ESAT-6 detection in CSF was more sensitive in comparison to conventional microbiological (smear/culture) and M-PCR tests for the diagnosis of cerebral tuberculomas. However, our results should be interpreted with caution because high background noise is often observed with I-PCR due to non-specific binding and the sample matrix effect, which leads to false positivity and thus compromise over the specificity of the test. This problem may be circumvented by the use of a liquid format using magnetic beads/gold nanoparticles-based I-PCR [9]. Nonetheless, this is the first report on the differential diagnosis of tuberculomas by I-PCR and appears promising for future use.

**Figure 2.** Multivoxel MRS of lesion at TE 144 revealed mildly increased Cho/Cr (choline/creatine) ratio of 1.35 with slight prominent Lip-Lac (lipid lactate peak), absence of alanine, succinate peak, and decreased NAA (N-acetylaspartate).
Conclusions

Differential diagnosis of CNS tuberculomas and NCC is crucial due to similar clinical and neuroimaging features. In this study, multiple ring-enhancing lesions of the brain by MRI, moderately high choline/creatine ratio by MRS, and the detection of Mtb specific ESAT-6 protein in CSF by ELISA/I-PCR seem to be useful modalities. Although I-PCR was efficient in this report, but it may not necessarily be a useful modality in other cases. Further work is warranted to validate the efficacy of I-PCR in a large number of samples.

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Conflict of interest

None.

References:

1. Manjunath MN, Bhakyalakshmi, Lakshmi et al: Multiple ring enhancing lesions in brain: Neurocysticercosis or tuberculoma? An extremely unusual/uncommon radiological presentation of a common disease: Central nervous system tuberculosis. Br J Med Pract, 2016; 9(1): a907
2. Yuzawa H, Hirose Y, Kimura T: A case of cerebral tuberculoma mimicking neurocysticercosis: A case report. Acute Med Surg, 2017; 4: 329–33
3. Rali P, Arshad H, Bihler E: A case of tuberculous meningitis with tuberculoma in nonimmunocompromised immigrant. Case Rep Pulmonol, 2016; 2016: 9016142
4. Mukherjee S, Das R, Begum S: Tuberculoma of the brain – A diagnostic dilemma. Magnetic resonance spectroscopy a new ray of hope. J Assoc Chest Physicians, 2015; 3(1): 3–8
5. Garg RK, Sinha MK: Multiple ring-enhancing lesions of the brain. J Postgrad Med, 2010; 56(4): 307–16
6. Shetty G, Avabratha KS, Rai BS: Ring-enhancing lesions in the brain: A diagnostic dilemma. Iran J Child Neurol, 2014; 8(3): 61–64
7. Ganesan R, Panchanathan S, Devi LU: Tuberculoma vs neurocysticercosis – a diagnostic dilemma. J Postgrad Med, 2016; 62(2): 82–83
8. Mehta PK, Raj A, Singh NP, Khuller GK: Detection of potential microbial antigens by immuno-PCR (PCR-amplified immunosassay). J Med Microbiol, 2014; 63(5): 627–41
9. Mehta PK, Dahuya B, Sharma S et al: Immuno-PCR, a new technique for the serodiagnosis of tuberculosis. J Microbiol Methods, 2017; 139: 218–29
10. Mehta PK, Kalta M, Khuller GK et al: Development of an ultrasensitive polymerase chain reaction-amplified immunoassay based on mycobacterial RD antigens: Implications for the serodiagnosis of tuberculosis. Diagn Microbiol Infect Dis, 2012; 72: 166–74
11. Mehta PK, Singh N, Dharra R et al: Diagnosis of tuberculosis based on the detection of a cocktail of mycobacterial antigen 85B, ESAT-6 and cord factor by immuno-PCR. J Microbiol Methods, 2016; 127: 24–27
12. Singh N, Sreenivas V, Gupta KB: Diagnosis of pulmonary and extrapulmonary tuberculosis based on detection of mycobacterial antigen 85B by immuno-PCR. Diagn Microbiol Infect Dis, 2015; 83: 359–64
13. Chatterjee S: Brain tuberculomas, tubercular meningitis, and post-tubercular hydrocephalus in children. J Pediatr Neurosci, 2011; 6(1): 96–100
14. Namani S, Dreshaj S, Berisha AZ: Tuberculous meningoencephalitis associated with brain tuberculomas during pregnancy: A case report. J Med Case Rep, 2017; 11: 175
15. Song F, Sun X, Wang X et al: Early diagnosis of tuberculous meningitis by an indirect ELISA protocol based on the detection of the antigen ESAT-6 in cerebrospinal fluid. Eur J Med Sci, 2014; 183: 85–88
16. Hasan Z, Jamil B, Ashraf M et al: Differential live Mycobacterium tuberculosis, M. bovis BCG-, recombinant ESAT6-, and culture filtrate protein 10-induced immunity in tuberculosis. Clin Vaccine Immunol, 2009; 16(7): 991–98
17. Sali M, Buonsenso D, Goletti D et al: Accuracy of QuantiFERON-TB gold test for tuberculosis diagnosis in children. PLoS One, 2015; 10(10): e0138952
18. Sharma S, Raj A, Singh N et al: Development of real-time immuno-PCR for the quantitative detection of mycobacterial PstS1 in tuberculosis patients. J Microbiol Methods, 2017; 132: 134–38