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Comparison of Real Time IS*6110*-PCR, Microscopy, and Culture for Diagnosis of Tuberculous Meningitis in a Cohort of Adult Patients in Indonesia

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Abstract

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Background

Bacteriological confirmation of tuberculous (TB) meningitis is difficult. Culture is slow and microscopy has insufficient sensitivity. We evaluated real time PCR targeting insertion sequence IS*6110* among 230 consecutive adult patients with subacute meningitis in a referral hospital in Indonesia.

Methods

Cerebrospinal fluid (CSF) samples were examined using microscopy, solid and liquid culture, and real time IS*6110*-PCR with a fluorescence-labeled probe using DNA extracted from CSF. CSF samples from 40 non-infectious neurology patients were used as negative controls. IS*6110*-PCR results were linked with clinical and CSF characteristics.

Results

Most patients presented with subacute meningitis, after a median of 14 days of symptoms (range 7–30). After exclusion of cryptococcal and bacterial meningitis, 207 patients were classified as definite or probable TB meningitis; 17.9% with HIV infection. Among this group IS*6110*-PCR gave the highest positivity rate (68%, 95% CI 62–74%) compared with microscopy of ZN-stained slides (11%, 95% CI 7–15%), and mycobacterial culture using solid (36%, 95% CI 29–42%) and liquid (44%, 95% CI 37–51%) media. IS*6110*-PCR was positive in 92% of patients with culture-positive and 42% of patients with culture-negative probable TB meningitis. Among culture-negative patients, a positive PCR was associated with a history of TB treatment, a longer duration of illness, a higher CSF cell count and

protein, and a lower CSF glucose. IS*6110*-PCR was negative in all CSF samples from non-meningitis control patients.

Conclusions

Real time IS*6110*-PCR is a quick, sensitive, and specific test for diagnosing of TB meningitis in this setting. Its performance in other (less-developed) settings needs further study.

Introduction

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Tuberculous (TB) meningitis is the most severe form of tuberculosis and causes substantial morbidity and mortality in adults and children [1]. Early recognition and treatment of the disease is believed to be able to reduce the burden of this disease, but this is hampered by the fact that it is often difficult to find bacteriological proof for TB meningitis [2], [3]. Microscopy of cerebrospinal fluid (CSF), although inexpensive and rapid, has a poor sensitivity, ranging from 1.9% to 20% in different series, with the exception of one study (58%) that used large volumes of CSF [3], [4], [5]. CSF culture, also lacks sensitivity for diagnosing TB meningitis [2], [3], [4], [6]. Furthermore, the slow growth of *Mycobacterium tuberculosis,* that usually takes up to 4 to 6 weeks limits the role of culture in decisions regarding initiation of TB treatment [7]. Therefore, a rapid and accurate diagnostic test would greatly benefit timely and adequate management of patients with possible TB meningitis.

Nucleic acid amplification (NAA) tests seem an attractive diagnostic tool for TB meningitis because of their speed and expected high sensitivity. However, a systematic review and metaanalysis of the accuracy of NAA tests for diagnosis of TB meningitis showed that commercial NAA test had a high specificity (98%, 95% CI 97–99%) but a low sensitivity when compared with culture (56%, 95% CI 46–66%) among 14 studies combined [8]. The recently developed GeneXpert system, combining DNA extraction with a real time PCR that simultaneously detects both *M. tuberculosis* and rifampin resistance, has a lower sensitivity compared to culture, but to our knowledge only one study has reported use in suspected meningitis [9]. In that study none of 19 CSF samples were positive with GeneXpert. This method was not available in Indonesia when the study was performed.

In-house PCR for diagnosis of TB meningitis may be more sensitive, possibly due to the use of nested PCR, DNA extraction methods, or use of different molecular targets. However, the precise role of in-house PCR for TB meningitis remains uncertain. Many in-house assays have been evaluated without adequate standardization and using small groups of patients [10], [11], [12], [13]. The present study therefore evaluated in-house real time PCR targeting IS*6110* in CSF samples from a well-characterized cohort of 230 adult patients with suspected meningitis, making comparisons with CSF *M. tuberculosis* culture and microscopy.

Methods

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Ethics Statement

Anonymized CSF samples were used from an already-existing hospital collection, collected as part of a project 'Optimization of diagnosis of meningitis', approved by the Ethical Committee of Hasan Sadikin Hospital/Faculty of Medicine of Universitas Padjadjaran, Bandung, Indonesia (No. 85/FKUP-RSHS/KEPK/Kep/EC/2006). The current study made use of an already existing sample collection, no separate patient consent was asked for this study. HIV testing is done routinely after verbal informed consent for all patients with suspected meningitis in Hasan Sadikin hospital. Consent is obtained from closest relatives (husband/wife or parents) for those patients who are unstable or unconscious at time of presentation. HIV testing was done anonymously afterwards for those who had died before consent could be obtained. This study was approved by the ethical review board of Padjadjaran University/Hasan Sadikin Hospital, Bandung, Indonesia.

Setting and Patients

Patients were recruited at Hasan Sadikin Hospital, top referral hospital for West Java, Indonesia, where