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Research Report

THE ANTI-TB DRUG SENSITIVITY OF *Mycobacterium tuberculosis* FROM CEREBROSPINAL FLUID AND BONE TISSUE BIOPSY SPECIMENS OF PATIENTS SUSPECTED TUBERCULOUS MENINGITIS AND SPINAL TB IN Dr SOETOMO HOSPITAL INDONESIA

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ABSTRACT

Tuberculous meningitis (TBM) is an infection of meninges which potentially life threatening with significant morbidity and mortality. Spinal TB has the same problem with TBM, infection in bone and joint, the delayed diagnosis worsens the prognosis. The rapid and accurate diagnosis plus prompt adequate treatment is essential for the good outcome. The aim of this research is to study the first line drug sensitivity of *Mycobacterium tuberculosis* isolated from specimens of cerebrospinal fluid from suspected tuberculous meningitis patients and bone tissue biopsy from suspected spinal TB patients. The method of this research is TB Laboratory examination in Department of Clinical Microbiology – Dr. Soetomo General Hospital, Indonesia, using the gold standard liquid culture method MGIT 960 System (Becton Dickinson) and solid culture method with Lowenstein-Jensen medium. The specimens CSF from 50 TBM patients at January 2013 until May 2014. Positive isolate detection of *Mycobacterium tuberculosis* complex were 11 isolates (22%), which sensitivity 100% (11/11 isolates) to Rifampin (R), Pyrazinamide (Z), Ethambutol (E), and Streptomycin (S); one isolate resistant to Isoniazid, sensitivity to Isoniazid 90,90% (10/11); and received 21 specimens of bone tissue biopsy which positive 5 isolates (23%), all isolates sensitive 100% (5/5 isolates) to Rifampin and Pyrazinamide, and 1 isolates resistant to Isoniazid, Ethambutol, and Streptomycin, in which sensitivity 80% (4/5 isolates) to Isoniazid, Ethambutol, and Streptomycin. The conclusion of this research is positivity detection 22% of CSF specimens, and 23% of bone tissue biopsy were low. All isolates sensitive 100% to Rifampin and Pyrazinamide, and 80-90% sensitive to Isoniazid.

Key words: first line anti-TB drug sensitivity, *Mycobacterium tuberculosis*, tuberculous meningitis, spinal tuberculosis, cerebrospinal fluid

ABSTRAK

*Meningitis tuberculosis (TBM) merupakan infeksi selaput otak/meninges, berpotensi mengancam kehidupan pasien dengan morbiditas dan mortalitas tinggi. Spinal TB juga memiliki masalah yang sama dengan TBM, yaitu infeksi pada jaringan tulang dan sendi serta kelambatan diagnosis yang memperburuk prognosis. Diagnosis akurat dan cepat, disertai segera pengobatan adekuat menentukan kesembuhan pasien. Tujuan penelitian ialah studi kepekaan obat anti-TB lini I di antara *Mycobacterium tuberculosis* complex isolat specimen cairan otak dari pasien diduga meningitis TB, dan biopsi jaringan tulang dari pasien diduga spinal TB. Metode penelitian ini ialah pemeriksaan laboratorium TB di Departemen Mikrobiologi Klinik/ RSUD Dr Soetomo, Indonesia, menggunakan metode gold standard metode kultur pada media cair MGIT 960 System (Becton Dickinson) dan metode kultur pada media padat Lowenstein-Jensen. Hasil penelitian ini ialah pada bulan Januari 2014 sampai dengan Mei 2014 diperoleh specimen cairan otak dari 50 pasien meningitis TB, terdeteksi 11 *Mycobacterium tuberculosis* complex (22%), sensitivitas 100% terhadap Rifampin (R), Pyrazinamide (Z), Ethambutol (E), dan Streptomycin (S) (11/11 isolat); satu isolat resisten terhadap Isoniazid, sensitivitas sebesar 90,90% (10/11) terhadap Isoniazid; pada 21 spesimen biopsi jaringan tulang ditemukan 5 isolat (23%), semua isolat 100% sensitif*

Rifampin dan Pyrazinamide, 1 isolat resisten terhadap Isoniazid, Ethambutol, dan Streptomycin, dengan sensitivitas sebesar 80% (4/5 isolat). Kesimpulan dalam penelitian ini ialah sensitivitas deteksi 22% dari spesimen cairan otak, dan 23% biopsi jaringan tulang, rendah, semua isolat sensitif 100% terhadap Rifampin dan Pyrazinamide, 80-90% sensitif Isoniazid.

Kata kunci: obat anti-TB lini I, *Mycobacterium tuberculosis*, meningitis TB, spinal TB, cairan otak

INTRODUCTION

Tuberculosis meningitis (TBM) is a common form of central nervous system infection in developing countries with high endemic TB. Delayed diagnosis and therapy are major factors in determining outcome, death or severe disabilities. Determining diagnosis of TBM based on the complementary standard examination of clinical manifestation, MRI/ cranial CT, cerebrospinal fluid (CSF) laboratory examination i.e. lymphocytes, glucose, protein, and microbes.¹

The definitive diagnosis of TBM based on isolation and identification of *Mycobacterium tuberculosis* from cerebrospinal fluid (CSF). Isolation and identification of *Mycobacterium tuberculosis* based on the clinical microbiology examination using the gold standard method as follow: culture method and PCR.^{1,2,3}

Developed early diagnosis of TBM such as PCR, GenXpert MTB/RIF, interferon-gamma release assay (IGRAs), tuberculostearic acid, and adenosine deaminase in CSF. Delayed diagnosis worsens the prognosis and increases morbidity. The microbiological diagnosis is crucial, despite surgical treatment always necessary anti-TB drugs (Merino *et al.*, 2012).⁴

METHOD

The 75 specimens or samples were CSF from suspected TBM and 21 bone tissue biopsy from suspected spinal TB patients received in TB laboratory of Department/ Instalation of Clinical Microbiology-Dr Soetomo General Hospital, Surabaya, Indonesia at January 2013 until Juny 2014.

Laboratory examination of clinical microbiology using the gold standard: liquid culture method MGIT 960 System (Becton Dickinson) and solid culture method with Lowenstein-Jensen medium; accurate specimens were centrifuged deposit of CSF or processed tissue to microbiologic examination.^{5,6,7}

RESULT & DISCUSSION

In TB laboratory of Department of Clinical Microbiology Dr Soetomo Hospital received 75 specimens CSF at January 2013 until Juny 2014. Positive isolate detection of *Mycobacterium tuberculosis complex* were 11 isolates (11/75 = 14,67%), which sensitivity 100% (11/11 isolates) to Rifampin (R), Pyrazinamide (Pza), Ethambutol (E), and Streptomycin (S); one isolate resistant to Isoniazid. Sensitivity to Isoniazid 91% (10/11) (Table 1).

Table 1. Positivity detection & first line anti-TB drug sensitivity of *Mycobacterium tuberculosis complex* isolate from CSF specimens of the suspect TB meningitis patients in Dr. Soetomo Hospital Indonesia, at January 2013-Juny 2014

| N | Specimen | Positive (%) | Sensitivity | | | | |
|----|-------------|------------------------|--------------|-------------|--------------|--------------|--------------|
| | | | R | I | Pza | E | S |
| 75 | Liquor/ CSF | 11 (11/ 75= 14,67%) | 11 (100%) | 10 (91%) | 11 (100%) | 11 (100%) | 11 (100%) |

R = Rifampin, I = Isoniazid, E = Ethambutol, S = Streptomycin, Pza = Pyrazinamide

At January 2013 until March 2014, TB laboratory- Department Clinical Microbiology - Dr. Soetomo Hospital received 21 specimens of bone tissue biopsy which positive 5 isolates (5/ 21 = 23.80%), all isolates sensitive 100% (5/ 5 isolates) to Rifampin and Pyrazinamide (Pza), and 1 isolates resistant to Isoniazid, Ethambutol, and Streptomycin. One isolate resistant to Isoniazid, Ethambutol, and Streptomycin, in which sensitivity 80% (4/5 isolates) to Isoniazid, Ethambutol, and Streptomycin (Table 2).

Positivity detection in this study is very low 11/ 75 CSF specimens (14,67%) positive isolate *Mycobacterium*

tuberculosis complex, and 5/21 bone tissue biopsy (23,80%) positive.

Many factors could influence the positivity detection of *Mycobacterium tuberculosis complex*, i.e. decided the appropriate criteria of clinical diagnosis for suspected TBM or for suspected Spinal TB; the accurate specimen for suspected TBM or for suspected Spinal TB related to paucy bacilli in locally tissue specimens; and specimen handling.

The sensitivity of *Mycobacterium tuberculosis complex* in this study revealed all isolates 11 from CSF and 5 from

Table 2. Positivity detection & first line anti-TB drug sensitivity of *Mycobacterium tuberculosis complex* isolate from bone tissue biopsy specimens of the patients suspected Spinal TB in Dr. Soetomo Hospital, Indonesia, at January 2013 - March 2014

| N | Specimen | Positive (%) | Sensitivity | | | | |
|----|--------------------|---------------------|-------------|------------|-------------|------------|------------|
| | | | R | I | Pza | E | S |
| 21 | Bone tissue biopsy | 5 (5/21=23, 80%) | 5 (100%) | 4 (80%) | 5 (100%) | 4 (80%) | 4 (80%) |

R = Rifampin, I = Isoniazid, E = Ethambutol, S = Streptomycin, Pza = Pyrazinamide

bone tissue biopsy 100% still sensitive to Rifampin and Pyrazinamide, and the other advantage were sensitivity 80-91% to isoniazid of isolates from CSF or bone tissue biopsy; isolates from CSF 100% sensitive Rifampin, Pyrazinamide, Ethambutol, and Streptomycin; isolates from bone tissue biopsy 80% still sensitive to Ethambutol and Streptomycin, otherwise the number of isolate samples were very small that could be not significant to reveal the conclusion on the sensitivity, need the valid research with multy centre study.

Accurate definitive diagnosis for TBM or spinal TB start with the essential step i.e. to determine the appropriate criteria standard for suspected clinically diagnosis, accurate specimen collection and handling, accurate standard method on laboratory examination for isolation and identification of etiologic *Mycobacterium tuberculosis*.

Accurate specimens for examination of Mycobacteria from suspected TBM patients: aseptic collection of 2-3 specimens of CSF with each volume 5-10 ml, because of paucy bacilli in CSF specimens.²

Accurate specimens for determine etiologic Mycobacteria from suspected spinal TB is bone and joint tissue biopsy durante operation or percutaneous biopsy guided by CT or MRI to obtain optimal tissues of destructive lesions, caseating granuloma or granulomatous inflammation or abscess in vertebral segments, 2 or more sites, on active cases could added blood aspirate around lesion with volume around 10 ml or more.^{8,9}

CONCLUSION

Determining tuberculous meningitis and spinal tuberculosis based on the gold standard that included the complementary of examination on clinical manifestation with the standard laboratory of the CNS characteristic figure on MRI/ CT; chronic inflammation or granulomatous or caseous necrotic on histo pathology; inflammatory reaction markers in blood, protein and glucose concentration, biochemical and pathological features in CSF on clinical pathology; and isolation and identification of etiologic bacilli *Mycobacterium tuberculosis* as definitive diagnosis.

Definitive Diagnosis based on isolation and identification *Mycobacterium tuberculosis* included the sensitivity to the first line anti-TB drug.

The gold standard method for isolation, identification, and sensitivity tests of *Mycobacterium tuberculosis* using the combined examination of standard culture method (solid and liquid medium) plus standardized PCR.

Positivity detection 14,67% of CSF specimens, and 23% of bone tissue biopsy were very low. All isolates 100% sensitive to Rifampin and Pyrazinamide, and 80% sensitive to Isoniazid, Ethambutol, and Streptomycin, with considered in very small isolate samples.

The important strategy need for better outcome in management TBM or spinal TB could be started by the research that included multy centre study to decide the standardized procedure on diagnosis, therapy, prevention and promotion.

Early accurate diagnosis and rapid appropriate therapy could be reached the better outcome, to ovoid disability sequele or mortality.

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