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Original Article

Mycobacterium Tuberculosis Identification on Suspected Extra Pulmonary Tuberculosis Patients

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ABSTRACT

Background: Extra Pulmonary Tuberculosis (EPTB) is Tuberculosis infection in organs other than the lungs, like pleura, lymph nodes, abdomen, genitourinary tract, skin, joints and bones, or meninges. The Diagnosis of EPTB is still challenging because symptoms of EPTB is vary, location EPTB is an inaccessible site, and paucibacillary smear. We wanted to know the Mycobacterium tuberculosis identification using the qPCR method on samples of EPTB suspects.

Methods: This research was a descriptive research with laboratory experiments. Subjects in this study were all cases of suspected EPTB with clinically diagnosed, thoraks radiographs, colonoscopy, and formalin fixed paraffin embedded (FFPE). DNA extraction was carried out according to PureLink® Genomic dna extraction kit instructions. The PCR volume 20 ul with 2x QuantiNova SYBR Green PCR Kit, M.tuberculosis Primer IS 6110, and DNA Sample. QPCR temperatures was done with predenaturation at 95 °C for 3 minutes, and followed 45 cycles, consist of denaturation at 95 °C for 15 seconds, annealing at 60 °C for 30 seconds dan extenstion at 72 °C for 30 seconds. PCR was performed with positive and negative controls, and data analysis was based on positive and negative ct controls.

Results: From 30 suspect EPTB samples, there are males (56,67%), with age 36-55 yo (50%), site of samples most from gastrointestines (70%), and there are 6 samples (20%) with positive qPCR.

Conclusion: qPCR methods can be used for EPTB diagnosis.

INTRODUCTION

Mycobacterium tuberculosis (Mtb) infection still become health problem in Indonesia. There are two clinical manifestations of Mtb infection such as pulmonary tuberculosis and Extra Pulmonary Tuberculosis. Pulmonary tuberculosis is the most common form. Extra pulmonary tuberculosis is a tb infection on other site except pulmonal, such as pleura, lymph gland, intestines, genitourinary tract, skin, joints, and meninges.[1–4] Extra pulmonary tb

cases 20,4% from all tb case, and lymph nodes were the most common organ to be affected organs in cases of EPTB.[5–7] Risk factor of extra pulmonary tb is young, female, asians and africans, and people with HIV.[8,9]

Establishing the diagnosis of extrapulmonary TB is still a challenge because clinical samples are difficult to obtain, or samples with few bacteria. This can reduce the sensitivity of diagnostic tests such as bacterial culture tests. Approximately 10 - 50% of extrapulmonary TB still involves the lung organ. Therefore, in all suspected cases of extrapulmonary TB, lung organ involvement must be assessed. In some cases of extrapulmonary TB, there is a positive sputum culture with normal radiographic results.

It is difficult to diagnose EPTB by using the conventional methods such as acid fast bacilli staining and culture, because it's less sensitive and culture takes longer time. For cases that from tissue in extrapulmonary tuberculosis, the diagnosis is made based on histopathological criteria for tuberculosis, tubercles consisting of caseous necrosis was found and granulomatous lesions are found, but it's may encompass many diffential diagnosis.[7,10–13] For cases of extrapulmonary tuberculosis that from fluid, it can provide a specific picture, if no specific picture is obtained, it cannot rule out that the case is not tuberculosis. EPTB samples from tissue with suspect tb presentation. EPTB with radiological positive test when there were pleural effusion, or tuberculomas. The clinical picture of extrapulmonary tuberculosis varies with nonspecific symptoms, making it difficult to diagnose, and also extrapulmonary TB samples are in the form of tissue/fluid that is sometimes difficult to reach, the number of extrapulmonary TB bacteria is few. This causes a delay in diagnosis with serious morbidity consequences. Early diagnosis of TB is crucial for effective management.[12,14,15]

For rapid diagnosis of extrapulmonary TB, PCR TB examination can be performed. The PCR method can detect a small number of bacteria, and extrapulmonary TB has few bacteria. PCR examination of extrapulmonary TB is very sensitive and specific for detecting MTB,[16,17] positive PCR results can be considered as a case of extrapulmonary TB, if the PCR TB result is negative, it cannot rule out the diagnosis of extrapulmonary TB.[18,19] Researchers want to know the Mtb identification in patients suspected of extrapulmonary TB using the qPCR method.

METHOD

This research was a descriptive research with laboratory experiments. The subjects of this study were all suspected cases of extrapulmonary tuberculosis that were diagnosed clinically, thoraks radiograph, colonoscopy, and histology. Extrapulmonary TB research samples obtained from July - October 2023 can be tissue/fluid/bone scrapings, and ffpe from suspected extrapulmonary TB patients.

DNA extraction

DNA extraction was carried out according to PureLink Genomic DNA Kit instructions and according to the type of sample obtained. 180 µl of lysis buffer solution and 20 µl of proteinase K were added to the sample into an Eppendorf tube, put into a waterbath for 1 h at a temperature of 55 ˚C, and room temperature for 3 minutes. Mixture added with 200 µl of purelink genomic lysis and vortexed, and add 200 ul 96% ethanol, vortex 5 s. The lysate prepared with PureLink® Genomic Lysis/Binding Buffer and ethanol to the PureLink® Spin Column and centrifuge at 10,000 × g for 1 minute at room temperature. The collection tube discarded and place the spin column into a clean PureLink® Collection Tube, and add 500 µL Wash Buffer 1 to the column, and centrifuge column at room temperature at 10,000 × g for 1 minute. The collection tube discarded and place the spin column into a clean PureLink® collection tube supplied with the kit. Add 500 µL Wash Buffer 2 to the column, and centrifuge the column at maximum speed for 3 minutes at

room temperature. Place the spin column in a sterile 1.5 mL microcentrifuge tube, add 80 µL of PureLink® Genomic Elution Buffer to the column, and incubate at room temperature for 1 minute. Centrifuge the column at maximum speed for 1 minute at room temperature. The tube contains purified genomic DNA, and store the purified DNA at –20°C until used.

MTB real-time PCR

PCR was performed by targeting 123bp fragment of insertion sequence IS6110 of MTB.[13,20–24] MTB primer F 5'-CCT GCG AGC GTA GGC GTC GG-3', R 5'-CTC GTC CAG CGC CGC TTC GG-3'. The PCR process began with 20 µl PCR reaction mixture, consist of 5 µl of Nuclease free water, 10 µl of MDx TaqMan 2X PCR Master Mix, 2 µl of M. tuberculosis Primer & Probe Mix, 3 µl of Sample DNA. In this study, the positive control was DNA of *M. tuberculosis* $H37R_V$ strain and negative control was RNAse Free Water. PCR amplification was done in Rotor-Gene Q 5plex (QIAGEN). PCR cycle I begins with an initial denaturation stage of 95°C for 3 minutes. PCR cycle II is carried out for 40 cycles. Each cycle consists of a denaturation stage at 95 °C for 15 seconds, primer attachment at 60 °C for 30 seconds. Interpretation of positive qPCR results based on the presence of a FAM filter (target detection), and a HEX filter (PCR validation). Negative qPCR results if there is no FAM filter (target detection), and there is a HEX filter (PCR validation). The Data is describe in tables.

RESULTS

Thirty Samples patients suspected of extrapulmonary TB were form pleural fluid, tissue biopsy and formalin fixed paraffin embedded. Based on the age of patients suspected of extrapulmonary TB, 50 percent of the EPTB samples were aged 36 - 55 yo with a ratio of male and female gender of 17:13 people. The location of the most extrapulmonary TB samples were from gastrointestinal biopsies.

Table 1. Characteristics samples EPTB

Variable		Number (n)	Percent (%)
Age			
Toddler	= 0 - 5 y.o	0	0
Children	= 5 - 11 y.o	1	3.33
Early adolescence	=12 - 1 6 y.o	1	3,33
Late adolescence	=17 - 25 y.o	3	1.00
Early adult	=26- 35 y.o	4	13.33
Late adult	=36- 45 y.o	7	23.33
Early ederly	= 46- 55 y.o	8	26.67
Late ederly	= 56 - 65 y.o	4	13.33
Ederly	= 65 – y.o	2	6.67
Gender			
Male		17	56.67
Female		13	43.33
EPTB Sample Location			
Pulmonary		5	19.23
Gastrointestines		21	76.92
Lymph node		3	3.85
Spines		1	0.00
qPCR Result			
Positive		6	23.08
Negative		24	76.92
Total			100

From 30 samples of suspected EPTB, there are 6 samples (23,08 %) detected MTB with IS6110 qPCR positive, which included 2 pleura fluids and 4 biopsy from colonoscopy. MTB qPCR has shown 23,08 per cent positivity in samples EPTB.

DISCUSSION

Diagnosis EPTB still challenging in the world, and requires high suspicion assisted by intensive examination.[1,6,18] The routine diagnostic methods have low sensitivity in diagnosis EPTB, due to few bacteria and the limitations diagnostic test. The gold standart diagnostic method need 6-8 wk for the report.[25] MTB PCR is a rapid diagnositic for MTB with specific target DNA sequences such as 65 kDa heat shock protein (HSP), IS6110 insertion sequences, gene coding for 38 kDa, 85B antigen and 16S rRNA.[20,26] IS6110 is specific for MTBC which 1 -20 copies per cell and dispersed in MTB genome. Amplication fragment targeted IS 6110 were 63 – 92 percent positive than other specific target DNA MTB.[10,20,21,23,27,28] IS6110 PCR can be highly useful in diagnosis of new and treated cases of EPTB.[23,29] The primer selection in this research in based on literature study that primer IS6110 is specific for MTB in EPTB cases, although Halse et al, qPCR MTBC with IS6110 is 5.7% for EPTB, it means that EPTB is more difficult to diagnosed.[28] PCR methods is sensitive, reliable, and easy to perform, it can be a tool in diagnosing EPTB beside of routine conventional diagnostic test dan clinical tb parameters.[30] PCR with more gene targets has increased the diagnositic yield of MTB infection, but PCR inhibitors, poor lysis of MTB during DNA extraction can give lower positivity and cross contamination can give false positivity.[12,24]

The diagnostic tests for EPTB are Ziehl Neelsen (ZN) microscopy, culture in LJ media, and BacT/Alert system, genexpert, and the gold standart test is culture MTB.[24,28]

A good diagnostic test must be done with control positive and negative and compared with gold standart test. In this research, we used one pair of IS6110 primer without compared with gold standart test. The limitation of this research is not included culture Mtb or ZN microscopy for compared the result qPCR, and pcr was done with one pair primer.

CONCLUSIONS

qPCR methods can be used for EPTB diagnosis, which is a useful tool in diagnosing TB in addition to routine conventional methods and TB clinical parameters. Further we can done qpcr multipleks MTB and microbiology comfirmed EPTB using MTB culture, or ZN microscopy.

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REFERENCES

1. *Abebe G, Deribew A, Apers L, Abdissa A, Deribie F, Woldemichael K, et al. Tuberculosis lymphadenitis in Southwest Ethiopia: A community based cross-sectional study. BMC Public Health [Internet]. 2012 Jul 6 [cited 2025 May 15];12(1):1–7. Available from: https://bmcpublichealth.biomedcentral.com/articles/10.1186/1471-2458-12-504*
2. *Ilgazli A, Boyaci H, Basyigit I, Yildiz F. Extrapulmonary tuberculosis: clinical and epidemiologic spectrum of 636 cases. Arch Med Res. 2004 Sep;35(5):435–41. https://doi.org/10.1016/j.arcmed.2004.05.008*
3. *Peto HM, Pratt RH, Harrington TA, LoBue PA, Armstrong LR. Epidemiology of extrapulmonary tuberculosis in the United States, 1993-2006. Clinical Infectious Diseases. 2009 Nov;49(9):1350–7. https://doi.org/10.1086/605559*

4. Tahseen S, Khanzada FM, Baloch AQ, Abbas Q, Bhutto MM, Alizai AW, et al. Extrapulmonary tuberculosis in Pakistan- A nation-wide multicenter retrospective study. PLoS One. 2020 Apr 1;15(4). https://doi.org/10.1371/journal.pone.0232134
5. Fairuz F, Syukri M, Devi Y. Geospatial Analysis to Identify the Area with High-risk Clusters of Extrapulmonary Tuberculosis in Jambi Municipality, Indonesia. journals.lww.com [Internet]. [cited 2025 May 15]; Available from: https://journals.lww.com/ijph/fulltext/2025/01000/geospatial_analysis_to_identify_the_area_with.28.aspx?context=latestarticles
6. JY Lee. Diagnosis and treatment of extrapulmonary tuberculosis. synapse.koreamed.orgJY LeeTuberculosis and respiratory diseases, 2015•synapse.koreamed.org [Internet]. 2015 Apr 1 [cited 2025 May 15];78(2):47–55. Available from: https://synapse.koreamed.org/pdf/10.4046/trd.2015.78.2.47
7. Fairuz F, H Dewi. Profil Ekstra Paru Tuberkulosis Secara Histopatologik Pada Formalin Fixed Paraffin Embedded (FFPE) Di Provinsi Jambi. repository.unja.ac.id [Internet]. [cited 2025 May 15]; Available from: https://online-journal.unja.ac.id/kedokteran/article/view/9476/5517
8. Niu T, He F, Yang J, Ma C, Xu J, Sun T, et al. The epidemiological characteristics and infection risk factors for extrapulmonary tuberculosis in patients hospitalized with pulmonary tuberculosis infection in China from 2017 to 2021. BMC Infect Dis. 2023 Dec 1;23(1). https://doi.org/10.1186/s12879-023-08410-w
9. Mehraj J, Khan ZY, Saeed DK, Shakoor S, Hasan R. Extrapulmonary tuberculosis among females in South Asia—gap analysis. Int J Mycobacteriol. 2016 Dec 1;5(4):392–9. https://doi.org/10.1016/j.ijmyco.2016.09.054
10. Sharma SK, Mohan A, Kohli M. Extrapulmonary tuberculosis. Expert Rev Respir Med [Internet]. 2021 [cited 2025 May 15];15(7):931–48. Available from: https://pubmed.ncbi.nlm.nih.gov/33966561/
11. Khan AS, Ali S, Khan MT, Ahmed S, Khattak Y, Abduljabbar, et al. Comparison of GeneXpert MTB/RIF assay and LED-FM microscopy for the diagnosis of extra pulmonary tuberculosis in Khyber Pakhtunkhwa, Pakistan. Brazilian Journal of Microbiology [Internet]. 2018 Oct 1 [cited 2025 May 15];49(4):909–13. Available from: https://pubmed.ncbi.nlm.nih.gov/29752010/
12. Sharma K, Sinha SK, Sharma A, Nada R, Prasad KK, Goyal K, et al. Multiplex PCR for rapid diagnosis of gastrointestinal tuberculosis. J Glob Infect Dis [Internet]. 2013 Apr [cited 2025 May 15];5(2):49–53. Available from: https://pubmed.ncbi.nlm.nih.gov/23853431/
13. Sharma K, Appannanavar SB, Modi M, Singh M, Sharma A, Varma S. Role of multiplex polymerase chain reaction using IS6110 and Protein b for the diagnosis of extra-pulmonary tuberculosis: North India. Indian J Pathol Microbiol. 2015 Jan 1;58(1):27–30. https://doi.org/10.4103/0377-4929.151164
14. Makeshkumar V, Madhavan R, Narayanan S. Polymerase chain reaction targeting insertion sequence for the diagnosis of extrapulmonary tuberculosis. Indian J Med Res [Internet]. 2014 Jan [cited 2025 May 15];139(1):161. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3994732/
15. Cherian JJ, Lobo I, Sukhlecha A, Chawan U, Kshirsagar NA, Nair BL, et al. Treatment outcome of extrapulmonary tuberculosis under Revised National Tuberculosis Control Programme. Indian Journal of Tuberculosis. 2017 Apr 1;64(2):104–8. https://doi.org/10.1016/j.ijtb.2016.11.028
16. Soini H, Musser JM. Molecular diagnosis of mycobacteria. Clin Chem [Internet]. 2001 [cited 2025 May 15];47(5):809–14. Available from: https://scholars.houstonmethodist.org/en/publications/molecular-diagnosis-of-mycobacteria
17. Hale YM, Pfyffer GE, Salfinger M. Laboratory diagnosis of mycobacterial infections: New tools and lessons learned. Clinical Infectious Diseases [Internet]. 2001 Sep 15 [cited 2025 May 15];33(6):834–46. Available from: https://pubmed.ncbi.nlm.nih.gov/11512089/
18. Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. FEMS Immunol Med Microbiol. 2012 Oct;66(1):20–36. https://doi.org/10.1111/j.1574-695X.2012.00987.x
19. Chakravorty S, Sen MK, Tyagi JS. Diagnosis of extrapulmonary tuberculosis by smear, culture, and PCR using universal sample processing technology. J Clin Microbiol. 2005 Sep;43(9):4357–62. https://doi.org/10.1128/JCM.43.9.4357-4362.2005
20. Negi SS, Anand R, Pasha ST, Gupta S, Basir SF, Khare S, et al. Diagnostic potential of IS6110, 38KDA, 65KDA and 85B sequence-based polymerase chain reaction in the diagnosis of Mycobacterium tuberculosis in clinical samples. Indian J Med Microbiol [Internet]. 2007 Jan 1 [cited 2025 May 15];25(1):43–9. Available from: https://pubmed.ncbi.nlm.nih.gov/17377352/
21. Narayanan S, Parandaman V, Narayanan PR, Venkatesan P, Girish C, Mahadevan S, et al. Evaluation of PCR using TRC4 and IS6110 primers in detection of tuberculous meningitis. J Clin

Microbiol [Internet]. 2001 [cited 2025 May 15];39(5):2006–8. Available from: https://pubmed.ncbi.nlm.nih.gov/11326036/

22. *Montenegro SH, Gilman RH, Sheen P, Cama R, Caviedes L, Hopper T, et al. Improved detection of Mycobacterium tuberculosis in Peruvian children by use of a heminested IS6110 polymerase chain reaction assay. Clinical Infectious Diseases [Internet]. 2003 Jan 1 [cited 2025 May 15];36(1):16–23. Available from: https://dx.doi.org/10.1086/344900*
23. *Maurya AK, Kant S, Nag VL, Kushwaha RAS, Kumar M, Dhole TN. Comparative evaluation of IS6110 PCR via conventional methods in rapid diagnosis of new and previously treated cases of extrapulmonary tuberculosis. Tuberk Toraks. 2011;59(3):213–20. https://doi.org/10.5578/tt.2680*
24. *Angmo D, Bashir G, Jan A, A. Khan M, Yasin SB. Multiplex PCR targeting IS6110 and mpb64 Genes for Detecting Extra-Pulmonary Tuberculosis: A Cross-Sectional Study in a Tertiary Care Center, Kashmir, India. Journal of Medical Microbiology and Infectious Diseases [Internet]. 2023 Jun 1 [cited 2025 May 15];11(2):78–85. Available from: http://jommid.pasteur.ac.ir/article-1-498-en.html*
25. *Schluger NW, Kinney D, Harkin TJ, Rom WN. Clinical utility of the polymerase chain reaction in the diagnosis of infections due to Mycobacterium tuberculosis. Chest [Internet]. 1994 [cited 2025 May 15];105(4):1116–21. Available from: https://pubmed.ncbi.nlm.nih.gov/8162735/*
26. *Tiwari V, Jain A. Application of enzyme amplified mycobacterial DNA detection in the diagnosis of pulmonary & extra-pulmonary tuberculosis - PubMed [Internet]. 2003 [cited 2025 May 15]. Available from: https://pubmed.ncbi.nlm.nih.gov/14870794/*
27. *Ogusku MM, Salem JI. Analysis of different primers used in the PCR method: diagnosis of tuberculosis in the state of Amazonas, Brazil. 2004 [cited 2025 May 15];30(4):433–9. Available from: https://www.jornaldepneumologia.com.br/details/1248/en-US/analysis-of-different-primers-used-in-the-pcr-method--diagnosis-of-tuberculosis-in-the-state-of-amazonas--brazil;*
28. *Halse TA, Escuyer VE, Musser KA. Evaluation of a single-tube multiplex real-time PCR for differentiation of members of the Mycobacterium tuberculosis complex in clinical specimens. J Clin Microbiol [Internet]. 2011 Jul [cited 2025 May 15];49(7):2562–7. Available from: https://pubmed.ncbi.nlm.nih.gov/21593269/*
29. *Raj A, Singh N, Gupta KB, Chaudhary D, Yadav A, Chaudhary A, et al. Comparative evaluation of several gene targets for designing a Multiplex-PCR for an early diagnosis of extrapulmonary tuberculosis. Yonsei Med J. 2016 Jan 1;57(1):88–96. https://doi.org/10.3349/ymj.2016.57.1.88*
30. *Siala M, Smaoui S, Taktak W, Hachicha S, Ghorbel A, Marouane C, et al. First-time detection and identification of the Mycobacterium tuberculosis Complex members in extrapulmonary tuberculosis clinical samples in south Tunisia by a single tube tetraplex real-time PCR assay. PLoS Negl Trop Dis [Internet]. 2017 May 1 [cited 2025 May 15];11(5):e0005572. Available from: https://pubmed.ncbi.nlm.nih.gov/28475618/*