Modern Mycobacterium Tuberculosis Strain in Bronchoalveolar Lavage from Tuberculosis Patients Associated with Lung Tissue Damage Severity

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Abstract

Background: The Beijing sublineage modern *Mycobacterium tuberculosis* strain is the most dominant strain in regards to causes of disease progression, extensive lung tissue damage, drug resistance and high outbreak rates.

Methods: *Mycobacterium tuberculosis* isolates were obtained from Bronchoalveolar lavage patients with active pulmonary Tuberculosis before obtaining anti-tuberculosis drug treatments. The degree of severity of parenchymal lung damage is classified by the NICE Scoring System. PCR was performed on DNA extracted from bronchial lavage, using primers targeting gene TbD1.

Result: 30 active pulmonary tuberculosis patients were analyzed in this study. 13 isolates of modern strains and 17 isolates of ancient strains were detected. In modern strains, 4 (30.8%) subjects had mild lung degree damage, while 9 (69.2%) subjects had severe lung damage. In ancient strains, 12 (70.6%) subjects had mild lung damage and 5 (29.4%) subjects had severe lung damage. *Mycobacterium tuberculosis* of modern strains correlated with the degree of lung damage, $p < 0.05$. Odds Ratio = 5.4 CI 95% (1,12-116,99).

Conclusion: In Surabaya, modern strains of *Mycobacterium tuberculosis* were detected in BAL of tuberculosis patients. Radiograph evaluations revealed severe lung tissue damage. The risk of severe lung damage with modern strains is 5.4 times higher than compared to ancient strains.

Keywords*: Modern Mycobacterium tuberculosis strain, lung tissue damage degree, bronchoalveolar lavage, Surabaya Indonesia.*

Introduction

In 2015, the World Health Organization (WHO) found 10.4 million cases of tuberculosis (TB) worldwide[1]. Indonesia is estimated to have one million new Tuberculosis (TB) cases each year. Currently, Indonesia ranks second of six countries with the highest TB cases in the world along with India, China, Nigeria, Pakistan and South Africa. By 2015, Through the WHO surveillance network [2], the WHO estimated that there were 10.4 million new TB cases, but only 6.1 million cases could be accounted for [3]. This appears to be due to *Mycobacterium tuberculosis complex* (MtbC) subspecies infection, especially those that infect humans, i.e. *Mycobacterium tuberculosis*. The determination of the tuberculosis strain from TB cases is necessary for observing the cause of extraordinary occurrences of tuberculosis at the local level, even in a larger range of regions [4].

Mycobacterium tuberculosis (MTB) divided into ancient and modern bloodlines [5]. The TbD1 deletion strain is also referred to as a "modern" evolutionary strain

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compared to the strain without deletion, which is known as the ancestral or ancient evolutionary form [6]. The global urgency related to the modern tuberculosis strain, just like Beijing strain or "W" strain, is correlated with the increase of TB infection risk and drug resistance. TB caused by the Beijing strain was observed in as many as 45.9% in Southeast Asia, 17.2% in Oceania and 16.5% in Middle East Asia[7].

In a diagnostic study, TB has been shown to have multiple forms. Generally, it is suspected as an active lesion including a cavity, miliary, infiltrate and fibroinfiltrate on the apical segment, posterior segment of the upper lobes, superior segment of the lower lobes and unilateral pleural effusion. The cavity illustrates the occurred disease expansion related to the degree of infection and the amount of *Mycobacterium tuberculosis* strains. It may increase the risk of TB infection to other susceptible individuals, accelerate spontaneous mutations that cause drug resistance, and be an independent risk factor for the recurrence of TB after obtaining adequate treatment [8].

Materials and Methods

This research was performed using a cross-sectional consecutive sampling method and through ethical feasibility tests in H. Moh Soewandhie Hospital, Surabaya Indonesia, from June 2017 until October 2017. 15 – 60-year-old patients with TB were diagnosed based on their sputum diagnostic tests and thorax radiographs. They did not receive anti-tuberculosis therapy and signed the informed consent form to join this study. The exclusion criteria included patients with HIV, diabetes mellitus, abnormalities in renal function, hearts and lungs. Ziehl Neelsen Acid Fast Bacilli (AFB) microscopic tests and result interpretations using the International Union Against Tuberculosis (IUALTD) scale was performed. The chest radiograph reading was performed independently by radiologists who were not aware of the patient's illness and the informed consent form. The degree of pulmonary parenchymal damage was classified using the NICE scoring system based on the lesion total in six lung areas [9].

Bronchoalveolar lavage was performed using ±50 cc in a sterile plastic bottle and placed in the refrigerator during the observation. Then, macroscopic analysis of the bronchial washing was observed. It could be decontaminated using the Petroff method, using NaOH with a 2:1 ratio. Next, it was centrifuged at 4000 rpm for 15 minutes. A cell pellet was obtained and PCR was performed to identify modern and ancient *Mycobacterium tuberculosis* strains with a TbD1 gen target. The positive control used was *Mycobacterium bovis*. To perform PCR, DNA was isolated from the decontaminated cell suspension of BA using a Qiagen KIT (1066955 01/2011) and mixed with 200µL Buffer Al and 20µL proteinase K by vortexing. 200µl (96%-100%) ethanol was additionally added and mixed.

Statistical Analysis

The collected data were processed using SPSS 17 software and analyzed with descriptive and analytical statistics. The correlation between the degree of lung damage and modern *Mycobacterium tuberculosis* strain infection on pulmonary TB group was analyzed with a chi-square test.

Result

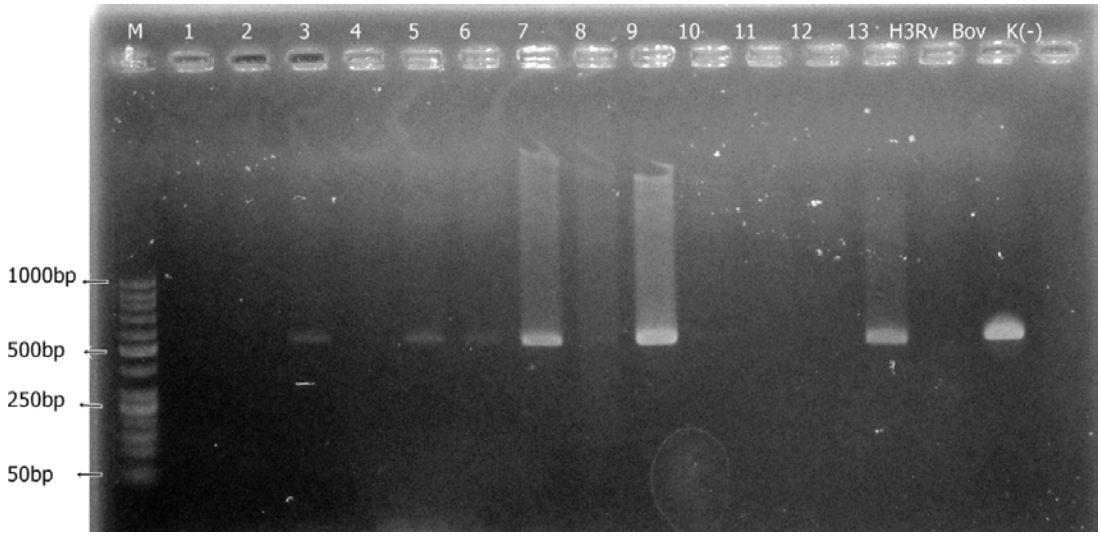
This study evaluated a total of total research subjects who had pulmonary TB but had not received treatment. These are the basic characteristics of research subjects.

Table 1. The basic characteristics of research subjects.

Characteristics		Frequency n (%)
Sex	Female	17 (56,7%)
	Male	13 (43,3%)

Cont... **Table 1. The basic characteristics of research subjects.**

Age	< 21 yo	4 (13,3%)
	21-30 yo	9 (30,0%)
	31-40 yo	6 (20,0%)
	41-50 yo	8(26,7%)
	>50 yo	3 (10,0%)
Education Backgorund	Elementary School (SD)	13 (43,3%)
	Junior High School (SMP)	7 (23,3%)
	Senior High School (SMA)	10 (33,3%)
Sputum Result	Negative	18 (60,0%)
	Positive	12 (40,0%)
Nodule	Yes	18(60,0%)
	No	12(40,0%)
Infiltration/ Consolidation	Yes	23(76,7%)
	No	7(23,3%)
Cavity	No	29 (96,7%)
	Yes	1 (3,3%)
Ectasis	No	3 (10,0%)
	Yes	27(90,0%)
Strain	Modern	13 (43,3%)
	Ancient	17 (56,7%)
Lung Damage Degree	Mild	16 (53,3%)
	Severe	14 (46,7%)



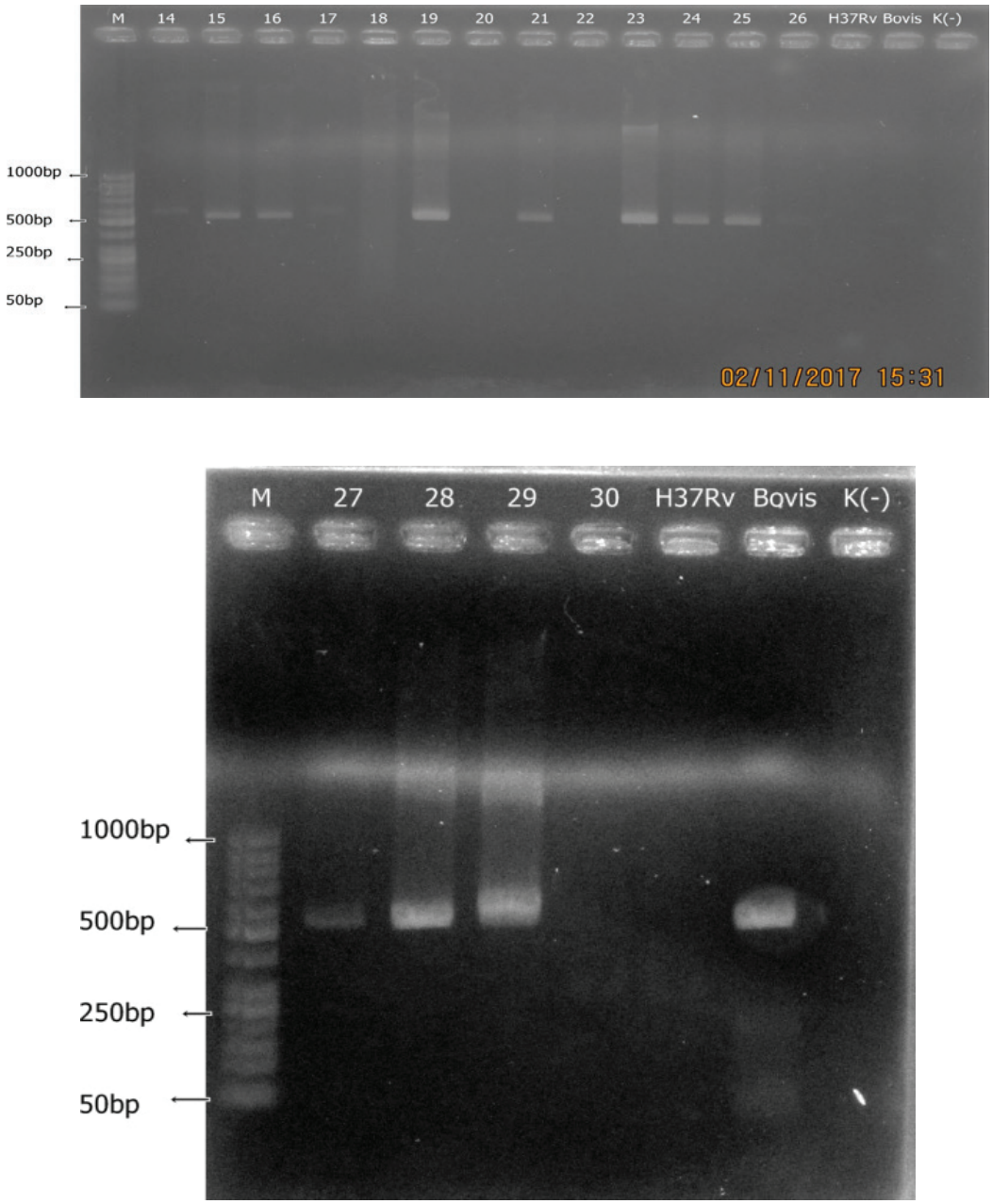


Figure 1. The results of electrophoresis gel using Tbd1.

In table 1 there are basic characteristics of the research subjects, the above analysis shows that the characteristics of the female sex are more frequent than males is 56.7%. at the age of 21-30 the greatest frequency is 30.0%. The background of primary school is the biggest frequency of 43.3%. The largest negative frequency sputum yield is 60.0%. Bintil who said yes the biggest frequency is 60.0%. Infiltration or Consolidation that says yes the biggest frequency is 76.7%. Cavity that says yes the biggest frequency is 96.7%. The ectasis that says yes the biggest frequency is 90.0%. Ancient tension is the largest frequency of 56.7%. The highest frequency of mild lung damage is 53.3%.

Table 2. The correlation between subject characteristics with *Mycobacterium tuberculosis*

Characteristics		Strain		Total	p-Value
		Ancient	Modern		
Age	<21 yo 21-30yo 31-40yo 41-50yo >50yo	1(25,0%) 4(44,4%) 5(83,3%) 4(50,0%) 3(100,0%)	3(75,0%) 5(55,6%) 1(16,7%) 4(50,0%) 0 (0,0%)	4 9 6 8 3	0,174
Sex	Female Male	8(47,1%) 9(69,2%)	9(52,9%) 4(30,8%)	17 13	0,225
Education Background	Elementary school Junior high school Senior High School	9(69,2%) 4(57,1%) 4(40,0%)	4(30,8%) 3(42,9%) 6(60,0%)	13 7 10	0,374
Sputum Result	Negative Positive	10(55,6%) 7(58,3%)	8(44,4%) 5(41,7%)	18 12	0,100
Nodule	Yes No	10(55,6%) 7(58,3%)	8(44,4%) 5(41,7%)	18 12	0,880
Infiltration/ Consolidation	Yes No	12(52,2%) 5(71,4%)	11(47,8%) 2(28,6%)	23 7	0,368
Cavity	Yes No	1(100,0%) 16(55,2%)	0(0,0%) 13(44,8%)	1 29	0,374
Ectasis	Yes No	14(51,9%) 3(100,0%)	13(48,1%) 0(0,0%)	27 3	0,238

Table 2 illustrates that there was no correlation between the subject characteristics (age, sex, educational background, sputum result, nodule, infiltration/ consolidation, cavity, ectasis) with *Mycobacterium tuberculosis* strain, $p>0.05$ for all characteristics were considered significant.

Table 3 The correlation between subject characteristics and Strain of TB Towards lung damage degree

Characteristics		Lung tissue damage		Total	P-Value
		Mild	Severe		
Sex	- Male - Female	7(53,8%) 9(52,9%)	6(46,2%) 8(47,1%)	13 17	0,626
Age	- <21 - 21-30 - 31-40 - 41-50 - >50	2(50,0%) 4(44,4%) 5 (83,3% 2(25,0%) 3(18,8%)	2(50,0%) 5(55,6) 1 (16,7%) 6(75,0%) 0 (0,0%)	4 9 6 8 3	0,104
Education Background	- Elementary school - Junior high school - Senior High School	5 (38,5%) 6 (85,7%) 5 (50,0%)	8 (61,5%) 1 (14,3%) 5(50,0%)	13 7 10	0,126
Sputum	- Negative - Positive	11 (61,1%) 5(41,7%)	7(38,9%) 7 (58,3%)	18 12	0,457
Nodule	- Yes - No	8(44,4%) 8(66,7%)	10(55,6%) 4(33,3%)	18 12	0,232
Infiltration/ Consolidation	- Yes - No	9(39,1%) 7(100,0%)	14(60,9%) 0(0,0%)	23 7	0,005
Cavity	- No - Positive	15 (51,7%) 1 (100,0%)	14 (48,3%) 0 (0,0%)	29 1	0,341
Ectasis	- No - Yes	3(100,0%) 13(48,1%)	0 (0,0%) 14(46,7%)	3 27	0,228
Strain	Lung Damage Degree		Total	p-Value	
	Mild	Severe			
Modern	4(30,8%)	9(69,2%)	13	0,035	
Ancient	12(70,6%)	5(29,4%)	17		

Table 3 Displays the correlation between subject characteristics and lung degree damage. There is a correlation between infiltration/consolidation and the degree of lung damage, p value<0.005. For the other characteristics, there was no correlation with lung damage, p value>0.005. Odds Ratio for Tbd1 (Modern Strain / Ancient Strain) = 5,4 CI 95% (1,12- 116,99). From 30 active TB patients, 13 isolates of modern strains and 17 isolates of ancient strains were obtained. From the 13 modern strains, there were 4 (30,8%) patient subjects with mild lung damage and 9 (69,2%) patients with severe lung damage. From 17 ancient strains, there were 12 (70,6%) subjects with mild lung damage and 5 (29,4%) patients with severe lung damage. There was a correlation between the *Mycobacterium tuberculosis* strain and degree of lung damage, p<0.005, OR= 5.4 CI 95% (1.12-116.99).

Discussion

Study in North India in 2014 found that 81.1% was the modern strain (TbD1-) and 24% positive *Mycobacterium tuberculosis* (ancient strain) [10]. This was due to the western location of Azerbaijan as the suburb of Iran with a larger population movement from neighboring countries [11]. An epidemiological study has reported that different genotypes of *Mycobacterium tuberculosis* were common in different geographical regions and the distribution of the genotypes is related to the migratory population [12]. [13] compared the TB genome based on the TbD1 deletion to differentiate each species of *Mycobacterium tuberculosis complex* (MTBC) into two major groups. 85.4% of modern *Mycobacterium tuberculosis* strain isolates (TbD1-) and 14.6% of ancient strain (TbD1+) was obtained. Modern strains have a low sensitivity phenotype of TB drugs with Relative Risk (RR) 0.89 (CI 95%, 0.74–1.07). In Mbarara, northern Uganda, 92.8% of the modern strain was found from 167 sputum samples of TB patients, so the TB epidemic in this area is commonly caused by the modern *Mycobacterium tuberculosis* strain [14].

The modern *Mycobacterium tuberculosis* strain has the following characteristics in radiological images: nodule (44.4%), infiltration / consolidation (47.8%), cavity (0%), ectasis (48.1%). The modern strain presents with mild lung damage, 4 patients (30,8%) and severe lung damage, 9 patients (69,2%). Ancient *Mycobacterium tuberculosis* strain has these following characteristics in radiological images: nodule (55.6%), infiltration/consolidation (52.2%), cavity (100%), ectasis (51.9%) with severe lung damage (29.4%). The ancient strain has mild lung damage 12 patients (70.6%) and severe lung damage 5 patients (29.4%). Odds Ratio = 5.4 CI 95% (1.120-26.044). A secreted protein, such as ESAT 6, is able to trigger macrophage death and is responsible for new macrophage withdrawal to form granulomas. It has an important role in granuloma expansion. ESAT 6 additionally triggers the MMP9 metalloproteinase matrix from macrophages freely, thus it may directly interact with epithelium. Epithelial cells around the infected macrophages also help to amplify MMP9 production. ESAT 6 mediates the induction of epithelial cells freely through TNF signaling and MYD88 pro-inflammation. In mice with *Mycobacterium tuberculosis* infections and devoid of MMP9, there was a small amount of macrophage withdrawal to lungs, decreased granuloma formation, and low amount of bacterial infection [15].

In experimental mice trials, there was a 40% occurrence of the cavity after *Mycobacterium tuberculosis* HN878 *(East Asian Lineage)* infection that was similar to the occurrence of the cavity on *Mycobacterium tuberculosis* H37Rv *(Euro-American Lineage)*. Caseous granuloma occurred in experimental mice with *Mycobacterium* virulent, i.e. *Mycobacterium Africanum,* with similar caseous lesions with the other experimental mice, which is infected by *Mycobacterium tuberculosis* with a different strain [16] [17] [18] showed that in C57BL/6 mice lesions of pulmonary necrosis induced by virulent *Mycobacterium tuberculosis* strain were observed, i.e. Beijing sublineae. Mice with *Mycobacterium tuberculosis* hypervirulent infection may have necrotic lesions in irreversible pulmonary.

The results demonstrated that the cavitary formation was not correlated with the Beijing strain infection in pulmonary TB patients [19]. [20] investigated and reported that there was no significant difference in the occurrence of the cavity in chest radiographs among the patients with Beijing and non Beijing strain infections. [21] Pulmonary TB patients infected with the W-Beijing strain has a very general radiograph. The *Mycobacterium tuberculosis* genotype is an independent factor for pulmonary tuberculosis radiograph. This research concluded that

active pulmonary TB patients in Surabaya were infected by the modern strain. The modern strain has a correlation to severe lung damage, p<0.05. Odds Ratio = 5.4 CI 95% (1.12-116.99).

Conclusions

In Surabaya, modern strains of *Mycobacterium tuberculosis* were detected in BAL of tuberculosis patients. Radiograph evaluations revealed severe lung tissue damage. The risk of severe lung damage with modern strains is 5.4 times higher than compared to ancient strains.

Conflict of Interest: There is no concflict of interest.

Source of Funding: This study is self-funded.

Ethical Clearance: This study was approved by Ethical Commission of Health Research Faculty of Medicine University of Airlangga in dr. Soetomo General Hospital Surabaya, Indonesia.

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