



Morphologic Profile Of Acid –Fast Mycobacterium Tuberculosis Bacteria In Patient Undergoing Four Months Treatment

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Abstract

Tuberculosis disease (TB) is a disease that is caused by *Mycobacterium tuberculosis*. Generally this bacteria is in perfect bar form, but sputum examination often indicates this bacteria is in uncommon form. The purpose of this study is to examine the differences of *Mycobacterium tuberculosis* morphology in patients' sputum every week for two months treatment.

This research used quantitative type with observational (non-experimental) research plan with time series analysis. Subjects for this research were five TB patients with positive acid resistance

bacteria observed from a sputum sample every week until eight week after by measuring the average of bacteria size and counting the percentage of bacteria degeneration.

The size of the bacteria continued to decrease with increasing treatment time each week. The size is reduced from 0.19 m - 0.49 m. The cell size decreased by an average of 46% with the smallest decrease at week 2 by 4% and the largest decrease at week six by 61%, before finally not being detected again at week eight. Patients with tuberculosis experienced a conversion or change from smear positive to smear negative after two months of treatment at the intensive stage. Bacterial cell damage was from week to week, increased by an average of 29% per week until finally negative at week 8 or two months after treatment. The highest level of damage occurred in the second week of 53%, and the lowest occurred in the sixth week of 15%, before finally 100% damage in the eighth week.

The conclusion of this study is that the size of *Mycobacterium tuberculosis* showed changes with the time of intensive treatment with the size reduced from 0.19 μ m - 0.49 μ m. The cell size was reduced by an average of 46%. Bacterial cell damage is from week to week with an average of 29% per week.

Keywords: Morphology: Bacteria: Fast Acid: *Mycobacterium*.

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Introduction

World Health Organization (WHO) and International Union Against Tuberculosis and Lung Disease (IUATLD) developed a TB control strategy known as the Directly Observed Treatment Short-course (DOTS) strategy. The DOTS strategy consists of 5 components. Of the five DOTS components, one of the most important is case finding through quality-assured microscopic examination of sputum carried out by health analysts. Microscopic examination of sputum not only serves to find TB cases, but also serves to provide an assessment of the health progress of TB patients. Currently the role of the laboratory is no longer to support the diagnosis but is to determine the diagnosis of TB (Anwar et al., 2018). The cause of TB disease is the mycobacterium tuberculosis. Mycobacterium tuberculosis is a bacterium that is Acid Fast Bacilli (AFB). AFB is a trait that is not only possessed by the bacterium of mycobacterium tuberculosis, but AFB is a trait possessed by the genus mycobacterium.

The morphology of mycobacterium tuberculosis bacteria is generally gram (+) rods, slightly bent, long or short stems (Velayati et al., 2018). However, based on the experience encountered by several health analysts when conducting microscopic examinations, it is often found that AFB has a different shape than its normal shape. Under the microscope, AFB often has a non-intact rod shape such as a dotted or shortened line (Qin et al., 2019). In Indonesia, patients diagnosed with TB disease are then given Anti-TB Drugs according to the national TB control program (Supriyana et al., 2019). The drugs include Isoniazid, Rifampicin, Prazinamide, and Ethambutol. Isoniazid (INH) has the action of inhibiting the formation of mycolic acid, a



component of the cell envelope (envelope) in mycobacteria. Rifampicin can cause inhibition of mycobacterial RNA synthesis. Pyrazinamide and Ethambutol are tuberculostatic to mycobacteria intracellular in macrophages (Meirelles et al., 2019). These drugs are the possible causes of damage to the mycobacterium tuberculosis bacteria, because these drugs are a nuisance to the mycobacterium tuberculosis bacteria. In the National TB Control Program, a good TB treatment is where two months after treatment there are no more smear-positive on microscopic examination of sputum preparations (Putri et al., 2014). However, there are some cases before two months negative but there are also those who are more than two months smear. Thus, it is possible that within the estimated time of one month to four months there will be changes in the morphology of the Mycobacterium tuberculosis bacteria before finally dying and becoming smear negative.

Based on the above background, researchers are interested in conducting research on the morphology of mycobacterium tuberculosis bacteria microscopically in patients with smear positive for four months of treatment.

Methods

The research was conducted at the Semarang Health Laboratory Center and the Health Analyst Department of the Health Polytechnic of Semarang. Samples (sputum) of patients who were examined and found to be smear negative. Positive AFB sputum were collected during the period of July to November 2020, then intensively treated with administration of anti-TB including Isoniazid, Rifampicin, Prazinamide, Ethambutol, and Isoniazid (INH).



Respondent' sputum taken were 18 people, who were newly diagnosed with TB during the period July to November 2020. Observation of microscopic morphology of Mycobacterium tuberculosis bacteria on sputum preparations of patients with positive smear for 2 weeks used Zeihl Neelsen staining (Erokhina et al., 2019). Gram staining was observed by using microscope multimedia Nikon 1000. Upon treatment, percentage of undamaged and damaged bacteria as well as changes in cell size length, width and dimensions (length x width) unit was analyzed by using microscope electron Hitachi 3000x. The morphology of Mycobacterium tuberculosis bacteria microscopically in the sputum preparations of patients with positive smear were examined 4 weeks after treatment. Percentage of undamaged and damaged bacteria was observed up to 10 weeks, carried out with a period of every 2 weeks. The morphology of the mycobacterium tuberculosis bacteria that has been observed using a microscope is documented by saving the photo, and counting percentage of the number of undamaged and damaged bacteria every two weeks. The course of bacterial morphology is studied to from time to time to obtain depth analysis.

Results and Discussion

The results of the examination data showed that 2 subjects (11%) were smear negative in the fourth week and on average at the sixth week of examination, there were an additional 9 patients with 50% negative smear results. On the eighth week all subject research has been declared negative (100%). The size is reduced from 0.49 μm to 0.19 μm . Cell size is decreased by an



average of 46% with the smallest decrease at week 2 of 4% and the largest decrease at week six by 61% before finally being undetectable in size again at week eight.

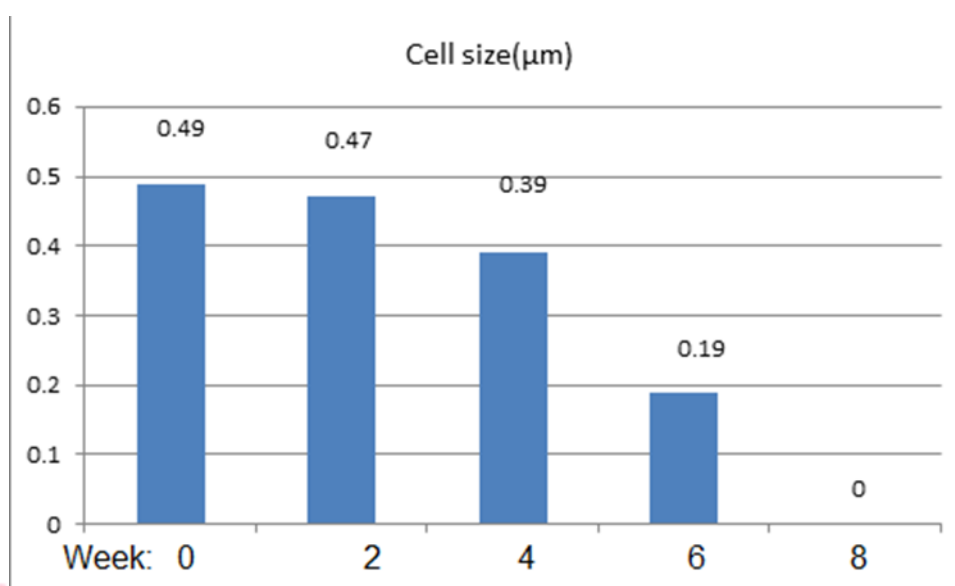


Figure 1. Cell Size of Mycobacterium tuberculosis bacteria during two months of treatment

Data on the level of cell damage in Figure 2 shows that Mycobacterium tuberculosis bacterial cell damage since week zero has occurred as big as 22.8% and tends to increase. At the sixth week the level of cell damage reached 85% and at the eighth week it is 100% damage.

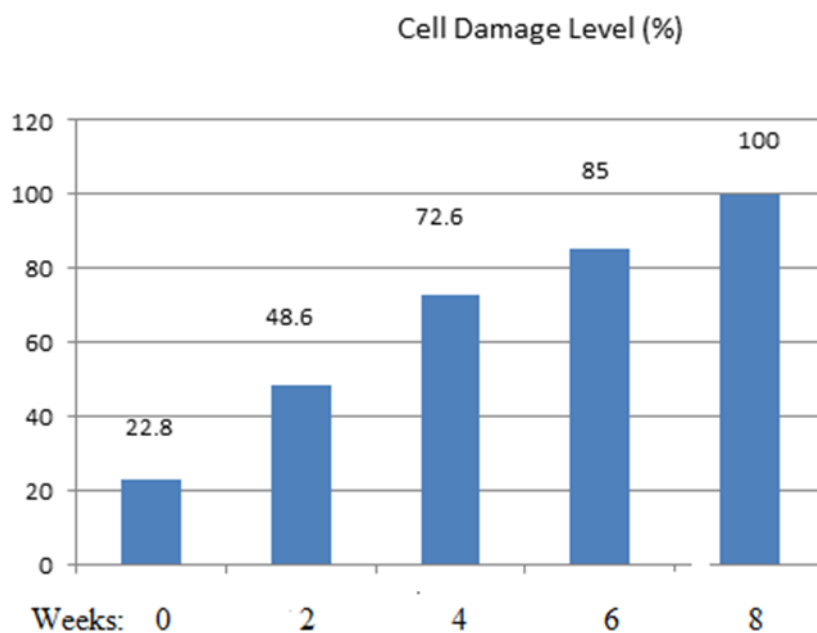


Figure 2. The level of damage to Mycobacterium tuberculosis bacteria

Data from the results of the examination showed that 2 subjects (11%) were smear negative at the fourth week and on average at week six the smear examination resulted in negative smears. On the eighth week all subject research has been declared AFB negative. These results provide empirical data that intensive, disciplined and regular treatment can produce the healing relatively fast. It can even be faster than 2 months (eight weeks). The Ministry of Health of the Republic of Indonesia states that a good TB treatment is where 2 months after treatment there is a conversion or change from positive smear to smear negative on microscopic examination of sputum preparations. So it is possible that within 2 months there will be a change in the morphology of the Mycobacterium tuberculosis bacteria before finally dying and becoming AFB negative.



Bacterial measurements were carried out by measuring the length and width of the Mycobacterium tuberculosis bacteria using a trinocular microscope expressed in μm . In the other hand the calculation of the percentage of damage to the Mycobacterium tuberculosis bacteria was also carried out simultaneously with the measurement of Mycobacterium tuberculosis bacteria. Thus, the researchers used the same field of view to measure and to calculate percentage damaged bacteria. The field of view used is 20 fields of view that are in the area of the longest horizontal line in the sputum preparation.

The data showed that the size of the bacteria continued to decrease in size with increasing treatment time each week. The cell size is decreased by an average of 46% with the smallest decrease at week 2 of 4% and the largest decrease at week six by 61% before finally being undetectable in size again at week eight. Cell size every two weeks from suspect to two months of treatment in the length range 1.07 m – 1.40 m and width range 0.19 m – 0.35 m. TB patients have undergone conversion or change from smear positive to smear positive. negative after two months of intensive treatment.

In this study, the sputum specimens used were sputum in patients with smear positive, then consume intensive anti-TB drugs. The drug is a RHZE tablet consisting of Rifampicin (R), Isoniazid (H), Pyrazinamide (Z) and Ethambutol (E) to be taken for 2 months.

Mycobacterium tuberculosis bacteria when experiencing a slight disturbance will be cut off into short rods and also have difficulty surviving if the patient gets anti-tuberculosis drugs (Raghuhandanan et al., 2018).



Mycobacterium tuberculosis bacteria have an inner compartment consisting of peptidoglycan (PG), arabinogalactan (AG), and mycolic acid (MA) which together form covalent bonds. These bonding strands are the main agents that function to maintain the shape of the bacteria (Velayati, 2018). One of the anti-TB drugs consumed by patients is Isoniazid. Isoniazid has the action of inhibiting the formation of mycolic acid, a component of the cell envelope (envelope) in mycobacteria (Vilch ze and Jacobs, 2019). Mycolic acid is one of the components in the main agent that functions to maintain the shape of bacteria. If the formation of mycolic acid is inhibited, the Mycobacterium tuberculosis bacteria will be easily damaged because there is nothing to maintain its shape.

The granules that appear in the Mycobacterium tuberculosis bacteria in the sputum preparations of patients with positive smear are also used as parameters in determining bacterial damage. As we have seen in the description above, most TB drugs destroy the Mycobacterium tuberculosis bacteria by attacking the bacterial cell wall. If the cell wall is damaged, the cell wall will peel off and look bacterial granules. Visible bacterial granules indicate that the bacteria have damaged the cell wall and what can be seen with a light microscope is the bacterial granules.

Conclusion and Recommendation

Morphological profile of Mycobacterium tuberculosis bacteria microscopically decreased the size of bacterial cells continuously starting from week 2 measuring 0.79 m to week 6 (0.19 m). The increase in bacterial cell damage averaged 29% with the level of damage in the 0 to 2 week period of 53%. In the end, at the eighth week it was 100% damaged and produced negative AFB.



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Routine treatment together with discipline and intensive care are the main mode to heal TB patients while maintaining the consumption of nutritious food. TB patients and their families should continue to do so in educating the persistence in intensive treatment phase.



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