

Original Article

The Immunoreactivity of Recombinant Protein MPT63 *Mycobacterium tuberculosis* as Serodiagnostic Tuberculosis

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ABSTRACT

Introduction: *Mycobacterium tuberculosis* protein-63 (MPT63) is an extracellular protein encoded by the Rv1926c gene with a size of 16 kDa. MPT63 can specifically induce macrophage activation, stimulate cytokine production (TNF- α and IL-6), and stimulate Th1 cells. The immunogenic properties of MPT63 can be concluded from the presence of a high density of T cell epitopes in the N-terminal immunodominant. This research aims to emphasize and determine the antibody response from the administration of the MPT63 antigen as a candidate in active TB serodiagnostic testing. **Methods:** This type of research is quantitative and is a diagnostic study. Serum samples were taken from Hasanuddin University Hospital stored biological material with 10 for healthy serum and 36 for sick serum. The determination of optical density values is based on the results of the ELISA reader. The determination of optical density values is based on the results of the ELISA reader. Sensitivity and specificity were tested using the Mc Nemar formula and then they calculated the sensitivity and specificity values manually. **Results:** Based on the interpretation of the ELISA test from the MPT63 antigen, the antibody reaction

response was obtained for a sensitivity of 50% and a specificity of 58.33%. **Conclusions:** Antibody responses to an antigen that is used indicate that all samples successfully respond well to the serum antigen in the serum of healthy and active tuberculosis but have a sensitivity that is still lacking.

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1. INTRODUCTION

Tuberculosis (TB) is a disease of lung infection caused by *Mycobacterium tuberculosis*. The onset of pulmonary tuberculosis disease is influenced by various factors. Epidemiological perspective has considered that the incidence of this disease can be seen as the result of the interaction between the three components, namely the host (host), causes (agent), and the environment (environment).^{1,2} The slow progress in dealing with bacterial infections makes the death rate still relatively high. Throughout the last decade of the 20th century, the number of new cases of TB is increasing worldwide, 95% of cases occur in developing countries. Data from the Global Tuberculosis Report in 2024 there were 10.7 million incident cases of TB which is equivalent to 140 cases per 100,000 population. The five countries with the highest incidence are India, Indonesia, China, Philippines, and Pakistan.³

Symptoms of TB are often not typical. The diagnosis is confirmed by finding the TB germ, but until now they do the best diagnostic search, quickly so that it can be a true gold standard category. Diagnosis using tuberculin test has many disadvantages, among which only determine that a person has been infected with TB germs, but cannot determine whether the TB infection is still ongoing or has been inactive, cannot distinguish whether a positive result occurs because the tuberculosis infection or due to BCG immunization and often occurs overdiagnosis followed by overtreatment.^{4,5}

It is expected that in the presence of a diagnosis method, others can instantly recognize the infection of *Mycobacterium tuberculosis*.^{4,6} Antibody tests for TB have long been sought, but serodiagnostic testing is a concern that interests to be studied more deeply.⁷ Utilizing specific *Mycobacterium tuberculosis* antigens and immunogenic in the serodiagnostic test could lead to the proper diagnosis and rapid and immediate treatment.⁸ Diagnosis of TB cases in recent years has been carried out by using some of the TB antigens that have been identified.^{6,9} One potential and immunogenic antigen is MPT63.¹⁰ Therefore, this research aims to emphasize and determine the antibody response from the administration of the MPT63 antigen as a candidate in active TB serodiagnostic. It is hoped that the results of serological examinations will show that the MPT63 protein can respond to antibodies so that it can be used as a candidate antigen for active TB serodiagnostic examination.

2. METHODS

This research has received ethical approval recommendations issued by the health research ethics committee of RSPTN Hasanuddin University and RSUP Dr. Wahidin Sudirohusodo Makassar with ethics approval number 238/UN4.6.4.5.31/PP36/2020. The MPT63 protein used as an antigen comes from the

MPT63 recombinant clone culture from previous research conducted by undergraduate students of the biology study program. Concentrations were made on the MPT63 protein and then the MPT63 protein stored biological material was taken. The protein was transferred to the new Eppendorf and PBS was added. A buffer coating was added to each new tube and then a protein sample was added. The sample is then vortexed and put into a well on the microplate and incubated for 2 days at -4°C . Wash buffer was added to all samples in the well. Washing is done 4x and the blocking solution is added and then incubated for 1 hour. Re-washing again as much as 4x using wash buffer. Store the plate at room temperature.

Serum samples derived from Hasanuddin University Teaching Hospital stored biological material comprised 10 serums from healthy people and 36 serums from patients with active TB. Samples were selected randomly by a purposive sampling technique. A new Eppendorf tube is taken with as many as 46 samples to be reacted with the protein MPT63 and then coded. Code S for T code for healthy and active tuberculosis. Figure 1 shows the serum sample to be added to the protein MPT63.

Samples and reagents were prepared and then laid layout well which will be added with the positive control, negative control, healthy serum samples, and active serum tuberculosis. A total of 46 empty Eppendorf tubes were filled with diluent samples and serum samples were added according to the code in the layout well made. The sample is then vortexed and inserted into each ELISA plate according to the layout well code. Positive and negative controls are also added to the layout. Cover the plate and incubate for 30 minutes at 37°C .

Washing was again done 3 times using wash buffer with an interval of each for 2 minutes then HRP- conjugate (1x) was added to each well, covered the microtiter plate, and incubated for 30 minutes at 37°C . Washed as much as 5 times using wash buffer. TMB substrate was added to each well and then incubated for 20 minutes at 37°C in the dark. The final step is to add Stop Solution to each well and cover the microtiter plate and then it is inserted into the ELISA reader. Determine the optical density value for each well by using a wavelength of 450 nm.

3. MAIN HEADING OF THE ANALYSIS OR RESULTS

Data from the optical density measurements are then processed by following the protocol of the ELISA Kit. TB antibody valence calculations for the detection of IgG compared with control samples. The cut-off value is defined as the value of the average negative control plus 0.10. The determination of IgG positive and negative responses from reactions that occur with the antigen is determined by looking at the ratio between the OD value of samples with the cut-off. If the sample $\text{OD} >$ cut-off value of the positive IgG response, otherwise if the $\text{OD sample} <$ value cut-off the negative IgG response.

Table 1. Results of Antibody Responses

Serum	Healthy		TB		Total
	IgG ⁺	IgG ⁻	IgG ⁺	IgG ⁻	
Protein MPT63	5	5	15	21	46

The results also showed that the protein table MPT63 used as an antigen can react at all whether it is healthy or serum in patients. Data from antibody response to an antigen is inserted into the table and the percentage of diagnostic test sensitivity and specificity values.

Table 2. Results of Antibody Responses

Antibody responses	Sample Group		Total
	Healthy	TB	
IgG ⁺	5 ^a	15 ^b	20
IgG ⁻	5 ^c	21 ^d	26
Total	10	36	46

4. DISCUSSIONS

Due to its specificity for complex TB, MPT63 has been studied by researchers for further research. Previous studies have identified potential microbacterial candidates for use as diagnostic tools, vaccines, or therapies. One such candidate is MPT63, which targets infected macrophages by regulating the expression of IFN- and ROS.¹¹

The release of IFN- γ on MPT63 T cells has also been used to distinguish TB patients from individuals infected with *M.avium*. Scientists have highlighted the diagnostic potential of MPT63 in healthy subjects who discuss BCG and PPD positive by detecting MPT63-derived CD4. CD4 T cell-specific epitopes T cell responses. Moreover, studies have demonstrated that MPT63 enhances the sensitivity of CFP-10 and ESAT-6-based diagnostic reagents.¹²

MPT63 may be involved in cell-host interactions to facilitate endocytosis or phagocytosis. Structural analogs with the highest scores. The structural similarity of MPT63 to immunoglobulin folds and proteins that coat the cell surface suggests host cell interactions and the ability to influence phagocytosis during bacterial internalization. This could potentially explain the protective nature of the anti-MPT63 immune response.^{13,14}

In-mold protein coating technology enables the transfer and integration of biologically active proteins to the surface of the polymer product microplate during the injection of polymer parts. The aim is to obtain a coating process that is very homogeneous in protein density. ELISA Kit is aimed at testing using solid-phase enzyme immunoassay (EIA) to detect the presence of ligands (proteins) in liquid samples using antibodies directed against the protein to be measured.

ELISA is one of the serological tests that can be used to detect the presence of antibodies. The main principle ELISA technique is the use of an indicator enzyme for immunological reactions. In this study, ELISA was used to detect IgG from serum samples of health and TB.

Colors are formed on an ELISA microplate reader to read on in to obtain the optical density (OD). The resulting OD values are proportional to the bound enzyme. Interpretation of the results of the antibody titer is expressed as the ratio of signal-to-noise (sn).

The results in Table 1 (Results of Antibody Responses) state that the amount of serum IgG⁺ response in healthy lower (5) the IgG⁻ response (5) as well as in serum IgG⁺ value TB (15) lower than IgG⁻ (21). Differences in DO value (optical density) due to

processes on their researcher's coating protein and not the result of the plant. This means that although the protein is attached to wells ELISA the number of different concentrations of the protein. The discovery of IgG⁺ value in the serum of patients was caused by the factor of serum samples that had long and often out-of-place incubation making the serum IgG⁺ on the decline.

The results of the diagnostic test found a 50% sensitivity for detecting which individuals are sick of the whole population of healthy serum. IgG⁺ was found in the group of healthy samples because of no assessment of TB so healthy samples that have IgG response possibility of having contact history. IgG⁺ which was found in a sample group of TB can be affected by various factors, some of which are in the serum sample storage duration of active TB, temperature influence due to the sample often being out of storage space and in addition, another possible factor because the recombinant protein used is not pure protein.

TB examination using MPT63 protein has fewer sensitivity values that need purification on a protein is better to increase the value of its sensitivity. Value specificity of 58,33% has adequate specificity value, but to raise the value of specificity to obtain a higher value again there should be selection criteria for both the fresh serum samples as well as samples of healthy patients.

5. CONCLUSION

Antibody responses to an antigen that is used indicate that all samples successfully respond well to the serum antigen in the serum of healthy and active tuberculosis but have a sensitivity that is still lacking.

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Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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