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IDENTIFICATION OF IGG AND IGM IN TB PATIENTS DURING 2 MONTHS OF TREATMENT USING SDS-PAGE

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. Several tests that can be performed to detect the presence of pulmonary TB are serological, one of which is, through the examination of immunoglobulin (Ig) G and M antibodies using the SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) method. This study aimed to determine whether IgG and IgM can be detected in TB patients during 2 months of treatment using SDS-PAGE. This study is descriptive by looking at the protein bands formed using SDS-PAGE. The results showed that all samples obtained IgM MW of 28.95 kDa; this protein's molecular weight (MW) indicates a humoral immune response in active TB patients. As for IgG, only the first sample obtained IgG MW of 157.97 kDa. This shows that the IgG MW, which is greater than the IgM MW, is formed after the patient has been on treatment for 2 months after experiencing TB infection and this IgG MW measure can last a long time even though the patient has recovered. Based on the results of this study, it is concluded that IgG and IgM in TB patients who have been on treatment for 2 months could detect IgG and IgM antibodies using SDS-Page. Thus, it is suggested for further researchers that the results of this study can be used as data and compared with TB patients who had 1 time exposed and 2 times exposed.

Keywords: *IgG, IgM, SDS-PAGE, Tuberculosis (TB)*

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis. Mycobacterium tuberculosis is transmitted through droplets in the air, so a tuberculosis patient is the main source of tuberculosis transmission. Treatment of this disease if not completed can cause dangerous complications to death. Until now, tuberculosis is still a health problem, both in the world and in Indonesia (Ministry of Health RI, 2016).

Routine tests for pulmonary TB examination such as sputum examination and conventional tuberculin test, which are often used in Indonesia, are still less sensitive. Based on some cases, often the bacteria in the sputum is not detected. In one study, out of 131 patients with pulmonary TB, only about 58.8% showed a positive sputum examination. Therefore, it is still necessary to find a diagnostic method that is easier and faster to do, simpler, and less expensive unlike the

diagnostic method with genetic amplification which is very complicated and expensive (Firmansyah. 2010).

Several other tests that can be done to detect the presence of pulmonary TB are serological. The principle of using the serological method is to detect the presence of antibodies in the patient's body. The sensitivity level is higher than the two traditional tests that are routinely performed, which is around 74.4%—and can be higher if the cut-off point is lowered (Ahmad et al., 2002).

Based on a study conducted by Makaminan et al. (2017), the IgG and IgM immunoglobulin antibodies in patients with suspected negative TB were smaller than those in positive TB patients. The primary immune response occurs when the antigen first enters the body, which is characterised by the appearance of IgM several days after being infected with germs or bacteria. IgM levels will increase for a short time, then slowly decrease and be replaced by IgG. The secondary immune response occurs when the antigen exposure occurs for the second time, which is also called a booster. Peak IgM levels in the secondary response generally do not exceed the peak in the primary response, on the contrary, IgG levels increase much higher and last longer.

Serological examination of the SDS-PAGE (Sodium dodecyl sulphate-polyacrylamide Gel Electrophoresis) method is a general method for identifying proteins and the results of protein purification based on their molecular weight. The working principle of SDS-PAGE is that when proteins are separated by electrophoresis through a gel matrix and an electric current is given, proteins with smaller molecules migrate faster while proteins with larger molecules are held back due to slower movement. Another influence on the rate of migration is based on the structure and load of the protein. The advantages of SDS-PAGE are that the migration process is faster, the spot separation becomes smaller by spectrophotometry and is easily dissolved in a small amount. Meanwhile, the disadvantage of SDS-PAGE is that there is OH interference in cellulose which can interact with polar molecules so the migration power of these molecules is disrupted and becomes lower.

Based on the background of the problem above, it is deemed necessary to conduct study on “Identification of IgG and IgM in TB patients during 2 months of treatment using SDS-PAGE at the Lepo-Lepo Community Health Center, Kendari. Some of the reasons considered are the economic level and accuracy of the examination, which are very necessary, especially in Indonesia.

METHOD

A. Research Type and Design

1. Research Type

This study is descriptive aiming to determine whether IgG and IgM can be detected in TB patients during 2 months of treatment.

2. Research design

The design used in this study was a descriptive laboratory by looking at the protein bands formed using SDS-PAGE.

The samples in this study were 2 TB patients who were actively seeking treatment at the Lepo-Lepo Community Health Center, Kendari.

B. Research procedures

a. Taking blood samples

1) Prepare necessary tools, namely a syringe, 70% kappa alcohol, a tourniquet, a plaster/bandage, and a tube.
2) Approach the patient in a calm and friendly manner, try to make the patient as comfortable as possible.
3) Attach the tourniquet about 10 cm above the elbow crease.
4) Select the median cubital or cephalic vein. Perform palpation to confirm the location of the vein.
5) Clean the skin, clockwise or circular.
6) Pierce the vein with a syringe at a 45 degree angle and the bevel is facing up.
7) When the needle has entered the vein, pull the holder until the blood fills the syringe as needed.
8) Remove the tourniquet, then gently remove the needle from the vein; use a dry cotton swab to apply pressure to the puncture site and then put a plaster/bandage.
9) Put the blood into the tube that has been prepared.

b. Centrifugation

1) To operate the appliance, plug it in at a voltage of 250.
2) Prepare the samples to be rotated and put them in a symmetrical and balanced place. When the samples preparation has been completed, they are centrifuged at 1500 rpm at 4^{o}C for 5-10 minutes.

3) Plasma is separated and transferred to a sample cup for further examination (Abunawas Hospital, 2017).

c. Plasma Sample Purification

1) Pipette 200 µl of sample into an eppendorf tube
2) Centrifuge at 2,500 rpm for 10 minutes at 4^{o}C
3) Add ammonium sulphate in a ratio of 1: 1
4) Centrifuge again at 1,400 rpm for 30 minutes at 4^{o}C.
5) Discard the supernatant, suspend the pellet with $\frac{1}{2}$ from the initial volume, then homogenise and store it in the freezer.

d. SDS-PAGE

1) Preparing samples

a) Plasma concentration is measured by using the Bradford or Lowry method.
b) After the concentration used is obtained, add a buffer sample, namely the added laemmli buffer sample (50 µl β-mercaptoethanol + 950 µl laemmli buffer sample), as much as 1:1.
c) Furthermore, the protein that has been added with the laemmli buffer sample is heated in a water bath or heat block (> 70^{o}C) for 3-5 minutes to denature the protein and accelerate the reaction in the analysed sample.
d) After the sample cools down, store the sample at 20^{o}C if it is not used immediately.

2) Making SDS-PAGE Gel

a) Prepare tools used to print gels such as spacer plates, short plates, and casting gel systems.
b) Assemble the tools used for making gel, by inserting spacer plates and short plates on the casting gel systems evenly to avoid leakage.
c) Preparation of 12% separating gel solution
d) Preparation of solution for 4% stacking gel

3) Running SDS-PAGE

a) Insert the gel into the chamber contained in the mini protein tetra cell, add a running buffer and then open the comb.

b) Protein samples and markers are inserted into the wells.
c) After protein samples and protein markers are inserted, close the mini protein tetra cell chamber. Connect the mini protein tetra cell cable to the power supply.
d) Run the gel at 100-120 V, for 60-90 minutes.
e) When finished, turn off the power supply, then lift the glass containing the gel and then remove the gel using the gel opener provided.
f) Verification of SDS-PAGE results can be done by staining with coomassie blue or coomassie Bio-safe.
g) After the colouring process, the gel destaining step is carried out.

After that, it can be visualised directly without using an imaging system (Fatchiyah, 2011).

RESULT AND DISCUSSION

1. Characteristics of Respondents

The characteristics of respondents based on age group can be seen in the table below.

Table 1.
Distribution of respondents by age (years)

No.	Age (Years)	Total	Percentage (%)
1	20	1	50
2	52	1	50
	Total	2	100

Source: Primary Data 2020

Table 4 shows that the 2 respondents in this study are 20 and 52 years old, with a percentage of 50% each.

2. SDS-PAGE Results

The image of the SDS-PAGE protein band with Coomassie Brilliant Blue staining on the serum of TB patients during 2 months of treatment can be seen in the image below.

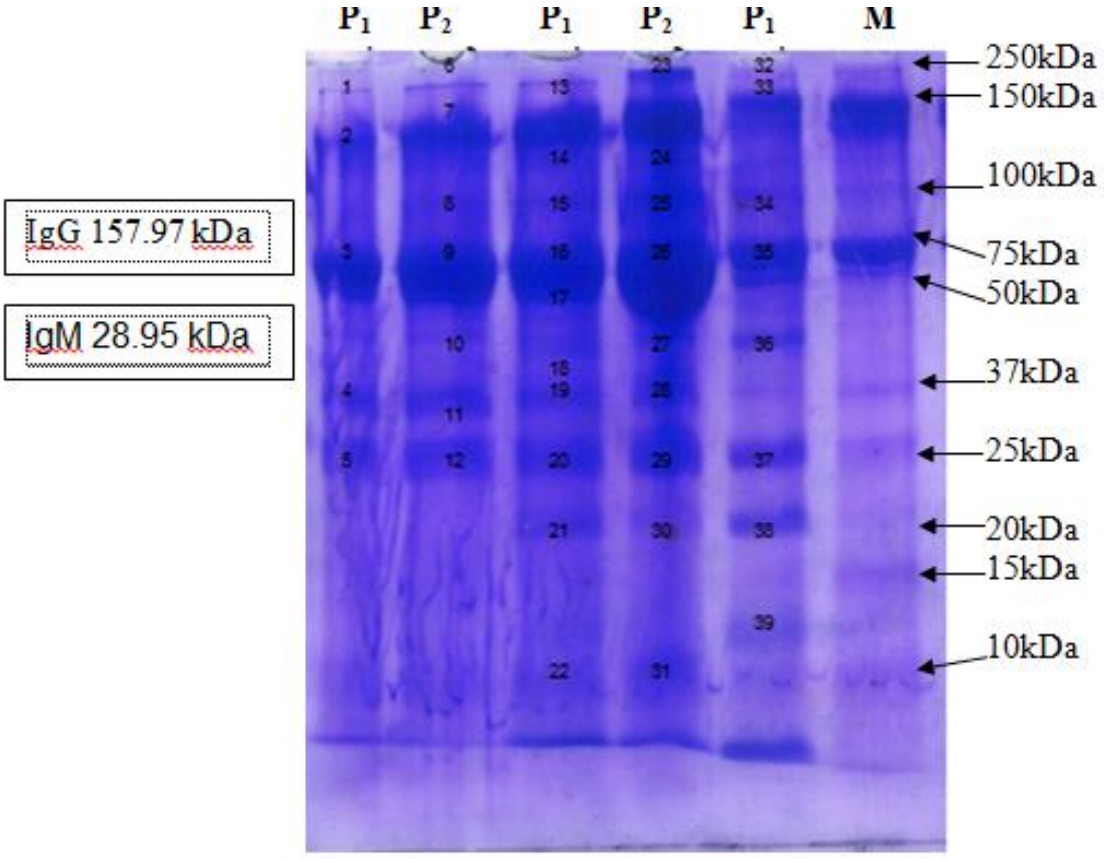

Figure 4. SDS-PAGE IgG and IgM protein results on the serum of TB patients during 2 months of treatment

Note:

Sample 1			Sample 2			Sample 1			Sample 2			Sample 1		
1	:	169.06	6	:	180.93	13	:	169.06	23	:	180.93	32	:	180.93
2	:	137.91	7	:	147.60	14	:	128.86	24	:	128.86	33	:	**157.97**
3	:	74.87	8	:	98.23	15	:	98.23	25	:	98.23	34	:	98.23
4	:	37.98	9	:	74.87	16	:	74.87	26	:	74.87	35	:	74.87
5	:	**28.95**	10	:	49.83	17	:	65.37	27	:	49.53	36	:	49.53
			11	:	33.16	18	:	40.65	28	:	35.49	37	:	**28.95**
			12	:	**28.95**	19	:	35.49	29	:	**28.95**	38	:	19.27
						20	:	**28.95**	30	:	19.29	39	:	12.82
						21	:	19.27	31	:	9.99			
						22	:	8.53						

TB treatment is given in 2 stages, namely the initial (intensive) stage and the advanced stage. The initial (intensive) stage is carried out during the first 2 months with the aim of reducing the number of germs in the patient's body. The initial (intensive) stage of treatment is also carried out to minimise the influence of a small number of germs that may have developed resistance to treatment before the patient received treatment. In the initial (intensive) stage, the patient receives medication every day, if this stage of treatment is carried out correctly, the infectious TB

patient becomes non-infectious within 2 weeks. Most smear-positive patients will become smear-negative within 2 months. Meanwhile, in the advanced stage, the patient received less medication. This advanced stage is useful to kill persistent bacteria so as to prevent a recurrence.

From the results of electrophoresis research conducted on serum samples of TB patients during 2 months of treatment, protein bands were obtained. Then the protein bands formed were calculated for length or Rf so that the molecular weights of the samples were obtained. In this study, 3 wells were made which functioned to insert/put the samples, the first well was filled with protein markers with a molecular weight of 10-250 kDa.

Based on the results obtained by IgG and IgM proteins carried out on 2 serum samples of TB patients during 2 months of treatment, it can be seen in Figure 4 that the separation by SDS-PAGE shows the presence of protein bands that are well visible, where all the bands formed have different thickness intensities. This shows that each serum protein has a different fraction. This is in accordance with a study conducted by Tanjung and Kusnadi (2014) that the thickness of the band (band intensity) formed indicates the concentration of protein in a compound, where the thicker the band, the higher the concentration.

The results of the examination found 39 protein bands from 2 samples where sample 1 was repeated triple and sample 2 was repeated double, each of which has a different molecular weight. This difference can be caused by improper pipetting. The existence of these differences according to Plummer (1979) is a result of differences in the primary structure of proteins because they are encoded by different genes. In addition, protein is a direct product of the nucleotide acid sequence of a gene, so the existence of these differences can be determined as a result of differences in genotypes between the cultivars tested.

The results of this study indicate that in all samples, 28.95 kDa of IgM was obtained, this result almost reached the IgM obtained by A-Rum et al. (2006), which was 29 kDa with a difference of 0.05, which indicates a humoral immune response. As for IgG, only sample 1 obtained IgG MW of 157.97 kDa. This result exceeded the molecular weight obtained by Flynn et al. (2001) which was 150 kDa with a difference of 7.97, which indicates a cellular immune response. The cellular immune response plays a role in the protection against intracellular fungi, viruses, and bacterial pathogens, while the humoral immune response is an immune response mediated by macromolecules found in extracellular body fluids called humoral immunity.

In relation to the results obtained with the age of the respondent, it can be said that the IgG of someone who is still of productive age (20 years old) is higher than that of a 52-year-old whose IgG has started to decline. This is in accordance with a study conducted by Fatmah (2006) that the function of the body's immune system (immunocompetence) decreases with age. The body's immune ability to fight infection decreases, including the speed of the immune response, as age increases.

In primary infection, IgM levels increase first, namely on day 3-5, while IgG levels increase on day 14. In secondary infection, IgG levels increase first starting on day 2, followed by IgM levels on day 5. However, the increase in IgG and IgM levels can vary from person to person. In some primary infections, IgM can persist in the blood for up to 90 days after infection, however, in most patients with infection, IgM levels will decrease and disappear by day 60.

IgM is the first immunoglobulin produced during infection. An increase in the amount of IgM reflects the presence of a new infection or the presence of an antigen. IgM is most active in the classical complement activation pathway. IgM molecules are bound by J chains. Most B cells contain IgM on the surface as antigen receptors. Meanwhile, IgG is the immunoglobulin that is formed after a few days of infection and can last a long time even though the patient has recovered.

CONCLUSION

Based on the study that has been done, it is known that all samples of TB patients who were treated for 2 months obtained IgM MW (molecular weight) of 28.95 kDa, which indicates a humoral immune response. As for IgG, only the first sample obtained IgG MW of 157.97 kDa which indicates a cellular immune response.

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