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Differences in Erythrocytes Sedimentation Rate Results using Physiological Saline Solution and Phopshate Buffer Saline in Pulmonary Tuberculosis Patients

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ABSTRACT

Tuberculosis (TB) is an infectious disease caused by the microorganism Mycobacterium tuberculosis (MTB) in the lungs. An inflammatory reaction occurs by phagocytic cells bye suppressing bacteria, as a result of this reaction there is a buildup of exudate fluid in the alveolus to form granulomas which are converted into fibtous tissue that can activate bacteria and then damage the lungs. NaCl 0,9% is the gold standard in Erythrocytes Sedimentation Rate (ESR) recommended by the Internatioanal Committee for Standardization in Hematology (ICSH). It was found that Saline Phosphate Buffer (PBS) has the same osmolarity pressuere as body fluids, and is isotonic so that it is similar to NaCl 0,9%. This study aimed to determine the difference in ESR results using PBS diluent pH 7,2 and pH 7,4 in tuberculosis patiens at the Blega Health Center, Bangkalan Madura regency. A total of 30 EDTA blood samples from tuberculosis patients. This study used an experimental method, with a static group comparation design on the difference in ESR results using NaCl 0,9% as the control group and PBS pH 7,2 and pH 7,4 as the treatment group, examined using the Westergren method. The results of tests using the One Way Anova test obtained a p- value 0f 0.997. the results of the Erythrocyte Sedimentation Rate using Phosphate Buffer Saline solution pH 7,2 and pH 7,4 with NaCl 0,9% in Tuberculosis patients. The conclusion of this study is that the results of ESR examination using PBS diluent solution pH 7,2 and pH 7,4 there was no difference in results with control group (NaCl 0,9%).

INTRODUCTION

Tuberculosis or often known as TB is a chronic infection that attacks the lung organs and one of the infectious diseases that are a problem throughout the world this infection is caused bye the bacterial spescies Mycobacterium tuberculosis (MTB), these bacteria are usually transmitted through droplet fluid of patients inhaled by prospective patients through the respiratory tract, besides that it can be infected through thr digestive tract and the presence of lesions on the skin (Sari et al., 2022)

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Doplet nuclei will enter through the nose, then into the upper respiratory tract, bronchi and into the alveolus. In the lungs bacteria will multiply and little by little will spread to the lymph glands. Inflammatory reactions are produced by the immune system by means of phagocytic cells will suppress the growth of bacteria and lymphocyte cells act as destroyers to lyse M. tuberculoisis bacteria. As a result of this reaction there is a buildup of exudate fluid in the alveolus. This stage is referred to as primary TB infection where at this time the patient has not shown symptoms and does not spread to others. While at the stage of secondary TB infection, bacteria will experience reactivation which can be caused by a decrease in the patient's immune system and the growth of bacteria that are getting stronger and will form granulomas. Granulomas are piles of living and dead bacilli surrounded by macrophage cells, which are then converted into fibrous tissue forming dormant bacteria where bacterial activation occurs and further damages the cells of the lung organs as a result of which the lungs become swollen and proceed to the diagnosis of bronchopneumonia (Iyah, 2021).

The Indonesian Ministry of Health in the Global TB Report stated that Indonesia was ranked second in tuberculosis cases after India with an estimated total incidence of 969,000 people, this figure increased by 17% compared to 2020. This equates to 11 deaths per hour or 93,000 deaths per year (Athosra et al., 2023). East Java Province ranks 2nd with the most TB cases after West Java province, in 2022 East Java recorded 81,753 or 74% of TB sufferers. The city with the highest TB cases occurred in the city of Surabaya with 10,741 cases found. Meanwhile, the TB achievement of Surabaya until March 2023 reached 14.25% (Hidriyah et al., 2018).

In TB patients there is a process of hypergammaglobunemia where globulin in the blood increases which then causes erythrocyte aggregation or rouleaux formation to increase faster so that to find out the value of blood sedimentation rate can be proven by LED examination to monitor the presence of disease infection. One of the test to monitor the severity of infection caused by Mycobacterium tuberculosis is Erythrocyte sedimentation rate (ESR). ESR is on of the examination in the body, generally ESR examination using NaCl0,9% diluent solution is the gold standard in ESR examination recommended by the International Committee for Standardization in Hematology (ICSH) which is isotonic and has the same osmosis tenure as body fluids (Ariyadi & Sukeksi, 2020).

In addition to NaCl, there is alternative reagent that can be used a blood thinner in ESR examination, namely Phopshate Buffer Saline (PBS) with a degree similarity of pH 7.2 and pH 7.4. Phosphate Buffer Saline (PBS) is one of the isotonic and non-toxic buffer solutions in cells containing disodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, and sodium chloride. PBS has the same properties as 0.9% NaCl solution so that it can maintain cell osmolarity pressure, maintain pH balance, and prevent cell damage (lysis) (Acording to Shofa in 2023,Amelia et al., 2013). PBS solution can be used as an alternative reagent in LED examination with a pH concentration close to NaCl 0.9% at PBS pH 7.2%. However, in the study, it is not yet known whether the results of erythrocyte deposits produced using PBS

solution will be the same if using abnormal patient blood samples. based on this explanation, researchers are interested in knowing the nature of PBS on ESR examination with samples from respondents with a history of pulmonary TB disease

MATERIAL /METHOD

This research was conducted at the Blega Health Center. The research method used was experimental with a Static Group Comparison approach.the study sample was tuberculosis patients using Purposive Sampling based on the length of treatment 0-3 monts and without a history of other diseases. the tool used is ESR reinforcement using Westergreen pipettes. The materials used in the study were 0.9% NaCl as the control group, PBS pH 7.2 solution and PBS 7.4 as the treatment group. The sample was examined using the Westergren method with a ratio of blood samples and diluent solution (1: 4), one as blood volume and 4 as diluent volume, allowed to stand perpendicularly for one hour, measured plasma height in the tube and expressed as ESR value in mm / hour. ESR value data is processed statistically, then tested for normality using Kolmogorov-Smirnov. Normal and Homogeneous distributed data followed by Anova One Way test. This study began on March 6 to April 24 at the Blega Health Center, Bangkalan Regency, Madura.

RESULTS AND DISCUSSION

Based on the results of research conducted at the Blega Health Center, Blega District, Bangkalan Regency from March 6 to April 24, 2024, the following results were obtained:

Tabel 1. ESR average test result of Wetergren method

Treatment	Mean (mm/jam)	SD	Min	Max
NaCl 0,9%	78,30	35,034	40	136
PBS pH 7,2	78.70	35,765	41	136
PBS pH 7,4	79,50	35,716	42	138

Based on table 1. showed that the results of descriptive tests and SD values of the Westergren method LEDs using 0.9% NaCl dilution were obtained on average 78.30 mm / hour with SD values of 35.034 mm / hour. The average result of the LED value using PBS pH 7.2 dilution is 78.70 with an SD value of 35.765, while the average result of LED values using PBS pH 7.4 is 79.50 with SD of 35.716 so that from these results it can be seen that the average result of LED values uses 0.9% NaCl dilution as a control group in this examination. LED values from 3 treatment groups between NaCl 0.9%, PBS pH 7.2 and PBS pH 7.4, are presented in figure 5.3 bar chart that the treatment group that is close to NaCl 0,9% value as a control group is LED value with PBS dilution pH 7.2.

Tabel 2. Anova One Way Results

ESR Resulst	df	Significance
Beetween Groups	2	0,997
Whithin Groups	27	

Based on table 2. The results of the One Way Anova statistical test on LED examination using a diluent solution of NaCl 0.9%, PBS pH 7.2 and PBS pH 7.4 obtained a significant value of 0.997 meaning a significance value of > 0.05. Then it can be known that hypothesis zero is accepted and hypothesis one is rejected. It can be interpreted that the results do not differ in LED values using PBS pH 7.2 and PBS pH 7.4 diluent solutions.

In this study, the results of data analysis using the Anova One Way test aimed to determine the differences between the control group and the treatment group, the requirement of this test is that the data must be normally distributed and homogeneous. Therefore, the first thing tested is normality, the normality test aims to find out that the data has been normally distributed on the basis of the decision of the significance result > 0.05, from this test obtained the significance value of the control group (NaCl 0.9%) and experimental group (PBS pH 7.2 & 7.4) >0.005, meaning that the data is declared normally distributed. From the results of homogeneity, a p-value of 1,000 is obtained, the result is known that the p-value > 0.05 means that the data is declared to have been distributed homogeneously. After the data is declared to have been normally distributed and homogeneous, the data is analyzed further on the Anova One Way test to determine the difference in 2 or more groups, from this result a significance value of 0.997 is obtained, meaning that the significance value is > 0.05, the results are presented in table 5.7. It can be seen that the Anova One Way test value > 0.05, then the null hypothesis (H0) is accepted and hypothesis one (H1) is rejected, meaning that there is no difference between the LED results using 0.9% NaCl diluent as the control group (independent variable) and the LED results using PBS pH 7.2 diluent and PBS pH 7.4 as the experimental group (dependent variable).

Stated that the results of LED examination using Phosphate buffer buffer solution saline (PBS) with pH 7.2 and 7.4 had no difference in results on LED examination. According to Diarti (2016) the use of Phosphate saline solution is isotonic, able to maintain pH, and does not cause lysis in cells when the solution is added, so that Phosphate saline solution is referred to as a buffer solution. It can be seen that among the 2 treatment groups that approached the control group was PBS solution pH 7.2. According to sabolakna (2022), blood samples that have been mixed with a pH 7.2 buffer solution have a specificity close to the control solution, namely 0.9% NaCl.

Erythrocyte Sedimentation Rate (ESR) is one of the parameters of hematology examination whose purpose is to detect and monitor tissue damage or inflammation in the body. Blood Sedimentation Rate (LED) is a laboratory test that is non-specific because LEDs only monitor the presence of inflammation or infection in the body and cannot detect a disease. Blood Sedimentation Rate (LED) measures how quickly erythrocyte cells settle in the

blood with certain anticoagulants within an hour and is expressed in millimeters per hour (mm/hour). LED examination is also often referred to as screening examination to monitor the presence of infectious diseases, autoimmune, diseases related to plasma proteins, and signs of malignancy (Nugraha et al., 2019).

The principle of ESR examination is that EDTA blood is diluted with 0.85% NaCl istonic solution or 3.8% sodium citrate anticoagulant in a ratio of (1: 4), 1 as the volume of anticoagulants and 4 as the volume of blood pipetted into a special LED tube (Westergren) in a perpendicular position within 1 hour, then erythrocyte cells will shed to the bottom of the tube due to differences in mass and type, Then it is calculated and expressed in mm / hour. The normal value of ESR for men is 0-15 mm / hour while for women 0-20 mm / hour.(Nugraha & Badrawi, 2018)

In TB patients there is a process of hypergammaglobunemia where globulin in the blood increases which then causes erythrocyte aggregation or rouleaux formation to increase faster so that to determine the value of blood sedimentation rate can be proven by LED examination to monitor the presence of infectious diseases, autoimmune, diseases related to plasma proteins, and signs of malignancy. There are several factors that can affect the results of a person's Blood Sedimentation Rate including, erythrocyte factors, plasma factors, and technical factors. This engineering factor is also the cause of erythrocyte cell deposition occurs faster (Athosra et al., 2023).

The selection of diluent solution on LED examination serves as a diluent for wholeblood samples. Diluent solution accelerates the formation of rouleaux due to increased plasma protein levels so that the attraction between red blood cells decreases and makes it easier for red blood cells to get close to each other. Diluent solutions commonly used in LED examination are NaCl and Sodium Citrate with a ratio of 1: 4, one is part of the diluent solution and 4 is part of the blood. Mismatch in the ratio of diluent solutions can affect blood concentration so that this can affect erythrocyte deposition (Nazarudin & Sari, 2021).

CONCLUSIONS

Based the results of the study of differences in the results of blood sedimentation rates using physiological saline solutions and saline buffer phosphate in tuberculosis patients, it can be concluded that: LED examination results using 0.9% NaCl diluent solution as a control group, namely 78.30 mm / hour. PBS pH 7.2 is 78.70 mm / hour. and PBS pH 7.4 which is 79.50 mm / hour. There is no difference in LED test results using PBS pH 7.2 diluent solution and PBS pH 7.4 with Westergren method ($p = 0.997$).

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