SURABAYA INTERNATIONAL HEALTH CONFERENCE
"Empowering Community For Health Status Improvement"
Novotel Samator East Surabaya Hotel, July, 13-14 2019

The Difference of Hemoglobin Examination Results in Normal and Hemolysis Samples Using Cyanmethemoglobin Methods

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Abstract: Hemoglobin examination is one of the most routine blood tests performed by each laboratory. Blood lysis is mostly caused by the breakdown of diplomatic blood cells. Erythrocyte lysis conditions silenced 15 minutes also can provide an effect on the cell membrane fragility. This study aims to analyze differences in hemoglobin examination results in normal samples and hemolysis using the Cyanmethemoglobin method. This type of research is an experiment. The study population was students and female students of Nahdlatul Ulama University in Surabaya with a sample size of 30 people taken by purposive sampling. Data retrieval is done through the provision of questionnaires and blood tests then analyzed using the One Way ANOVA test, α=0.05. The results showed that of the 30 D-IV Students of Medical Laboratory Technology had an average value of Hb in normal erythrocytes as a control ie 13.1 g/dl, the average Hb level with treatment of lysis erythrocytes without incubation was 13.8 g/dl, the average Hb level with the treatment of lysis erythrocytes with 15 minutes incubation is 12.8 g/dl and the average hemolysis is 1.2%. The results of the analysis through the One Way ANOVA test show that ρ=0.097 which means $\rho>\alpha$, so that H_0 is accepted. So it can be concluded that there is no difference in the results of hemoglobin examination between normal samples and hemolysis in the blood to the routine examination used.

Keywords: Hemolysis, Hemoglobin, Cyanmethemoglobin.

1. Introduction

The laboratory can be said to be of high quality if the data produced by the laboratory can satisfy patients by paying attention to a technical aspect such as high precision and accuracy and the data must be well documented so that it can be maintained scientifically [(1)]. Errors in the pre-analytic process can contribute 61% of total laboratory errors [(2)].

Lysis blood, also known as hemolysis, is an event of bloating and destruction of blood cells due to the entry of water into the erythrocytes so that hemoglobin exits from the erythrocytes into the surrounding fluid due to incorrect sample preparation [(3)]. Blood lysis is mostly caused by plasma red blood cell breakdown [(4)].

Storage time can significantly affect the decrease in the number of erythrocytes, total leukocytes, neutrophils and lymphocytes. The longer the storage, the number of cells counted also decreases because the cells are damaged (hemolysis) or die. During storage, blood cells experience changes include changes in biochemical, biomechanical, and immunological reaction, which causes damage to structural/morphological known as storage lesion. Most in the laboratory the condition of erythrocyte lysis is still used as an examination material for certain reasons, such as patients refusing to take blood again, doctors want laboratory results as soon as possible, and many others [(4)]. As a small part of the blood lysis incubated for several minutes after centrifugation can separate erythrocyte cells from plasma so that it can minimize an examination result. Erythrocyte lysis conditions silenced 15 minutes also can provide an effect on the cell membrane fragility [(5)].

The usefulness of this hemoglobin examination is to determine whether there is a health disorder in a patient. Hemoglobin is the main component of red blood cells or erythrocytes consisting of globin and heme consists of a porphyrin ring with one atom of iron (ferro). Globin consists of 4 polypeptide chains in the form of 2 alpha (α) 2 and 2 beta (β) polypeptide chains 2. Alpha polypeptide chain

consisting of 141 amino acids and beta polypeptide chains composed of 146 amino acids. Normal hemoglobin in the blood of an adult consists of Hb A (96-98%), Hb F (0.5-0.8%) and HbA2 (1.5-3.2%) [(6)].

In this study I took a hemoglobin examination using the cyanmethemoglobin method as a standard examination in a clinical laboratory, using a spectrophotometric device. according to the International Committee for Standardization in Hematology (ICSH) has determined that the gold standard of hemoglobin currently using methods cyanmethemoglobin [(7)]. This is because the examination of the hemoglobin spectrophotometer method has a smaller error rate [(6)].

The principle of examination of cyanmethemoglobin is heme (ferro) oxidized by potassium ferrisianide to (ferric) methemoglobin then methemoglobin reacts with cyanide ions to form cyanmethemoglobin which is brown, absorban is measured by colorimeter or spectrophotometer at a wavelength of 540 nm [(6)].

One of the most influential factors on the results of the examination is preanalytic such as blood lysis [(8)]. Disorders due to blood lysis in laboratory measurements are caused by many factors, namely the release of intracellular substances in the blood, microscopic interference as well as the release of active substances that can interfere with and trigger laboratory reactions [(9)]. The level of blood samples rather lysis and lysis on the use of blood samples can influence the results of hemoglobin examination [(6)]. Because the greater the hemoglobin released from the erythrocytes is lysis, then the calculated levels and absorption of the absorbance is also getting bigger [(7)].

The purpose of this study was to analyze differences in hemoglobin examination results in normal samples and hemolysis using the Cyanmethemoglobin method.

The results of this study are expected to provide scientific evidence and application of the correct examination flow regarding differences in hemoglobin examination results in normal samples and hemolysis using the Cyanmethemoglobin method.

2. Research Methodology

2.1. Research methods

This study is a laboratory experimental research with pretest-posttest design with a control group. The population in this study were students and students of the Nahdlatul Ulama University in Surabaya.

The research sample was D-IV Students of Medical Laboratory Technology, Nahdlatul Ulama University of Surabaya with the criteria for inclusion sample namely D-IV Students of Medical Laboratory Technology UNUSA who was active, registered as UNUSA Student by having KTM data (Student Identity Card), and willing to take blood.

The sample size is calculated by the formula Federer [(11)], for experimental tests. Based on the calculations that have been done obtained the minimum number of samples that will be done research that is equal to 14 samples. So for the overall sample used in this study as many as 28 people or sample with given two treatments, So that to facilitate testing of the statistical data analysis of samples rounded up to 30 samples.

2.2. Time and Place

The research was conducted at the UNUSA Laboratory, B Tower campus, on Jl. Raya Jemursari no. 57, Jemur Wonosari, Wonocolo, Surabaya city, East Java. From February 26 to April 6, 2019.

2.3. Tools and Materials

The tools used in this study were UV-Vis spectrophotometer, cuvette, tourniquet, centrifuge, serological tube, label, serological tube rack, 50 µl micropipette, 20 µl micropipette, yellow tip, suction ball, vacuntainer tube, 5 ml volume pipette, 3 ml syringe, wing needle, 100 ml beacker glass, 250 ml beacker glass, 250 ml volumetric flask.

The material in the study consisted of venous blood from the patient, 70% alcohol, tissue, lisol, 0,85% NaCl, 0,10% NaCl dilution and Drabkins reagent.

2.4. Procedure

Sample Preparation, Processing of Test Materials (samples taken on the arms of the cubic vein section as much as 3 CC placed into the EDTA tube), Determination of Cyanmeth Hemoglobin Standard

Replacement Factors (respondent samples were taken at the Clinical Laboratory to determine Hb levels in units of g / dl, after that sample brought into the UNUSA Laboratory with the help of icegell in order to measure the absorbance with a wavelength of 540 nm using a spectrophotometer), the technique to lyse the sample on Hemoglobin examination in the blood (the sample taken transferred to the EDTA tube is divided into two parts, one tube that contains The venous blood was hardened 30 times, then the sample was damaged again with the help of sticks in the middle area of the sample quickly, then to accelerate the thinning assisted by a spectrophotometer at 4000 rpm for 10 minutes).

Then the procedure was performed from 3 treatments and read the Hb level using a formula that is the absorbance level multiplied by the factor results. and the percentage calculation obtained from lysis absorbance using 0,90% NaCl solution divided by lysis absorbance using 0,10% NaCl solution then multiplied by 100%. after that the recording of the results of data checking and processing is carried out, and data analysis uses the IBM SPSS statistics 23 version (IBM corporation) with the One Way ANOVA Test, with a confidence level of 95% which means α=0,05.

3. Result and Discussion

Respondents taken In this study, 30 respondents each carried out 3 treatments, not with the same gender characteristics because of the limitations of respondents from D-IV female students Health analysts themselves were dominated by women who could be proven through real data from the Administration D-IV Study Program Health Analyst. The percentage of gender characteristics in the respondents can be seen in Figure 1.

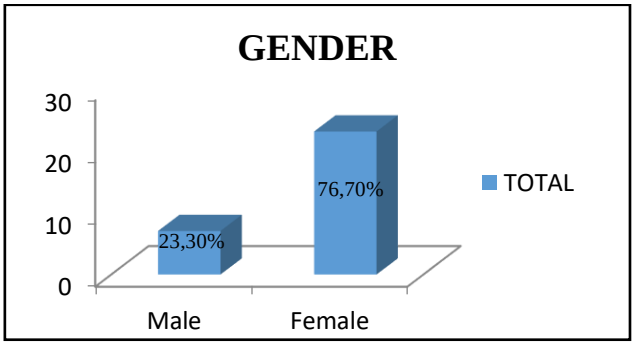

Figure 1. Percentage of gender characteristics in the respondents

According to previous researchers, gender greatly influences hemoglobin levels, because every month women experience menstruation so that it can allow a woman to experience a decrease in hemoglobin levels than men. According to Istiany and Rusilanti in 2013, women were more prone to decreased hemoglobin levels compared to men, which for the first reason was because every month women experience menstruation, women often menstruate during their childbearing years and during menstruation women experience bleeding, and iron deficiency due to bleeding can be overcome with adequate iron intake and iron supplementation.

After obtaining the results of a factor of 35,75 and the results of absorbance measurements on hemoglobin examination, the calculation will be continued using a predetermined formula so that the average measurement results of Hb levels in the gr/dl concentration and SD values of 3 treatments can be seen in Table 1

Table 1. Results of Mean ± SD from Measurement of Hb Level

Group Treatment	TOTAL	Mean ± SD	P *Value*
Normal erythrocytes (control)	30	13,137 g/dl ± 1,7713	0,097
Lysis Erythrocytes Without Incubation	30	13,798 g/dl ± 1,8637	
Lysis Erythrocytes With 15 Minutes Incubation	30	12,787 g/dl ± 1,8112	
Total	90	13,241 g/dl ± 1,8442	

Based on the research data in Table 2 shows that the distribution of Hb levels with initial treatment where blood samples were examined Hb with normal erythrocytes (without lysis) as controls

in 30 respondents with an average regardless of the sex of 13,137 g/dl ± 1,7713 . Hb level distribution with the second treatment where blood samples were done by erythrocyte cell extraction without incubation and then measured hemoglobin in 30 respondents with an average without distinguishing the sex of 13,798 g/dl ± 1,8637.

Distribution of Hb levels with the third treatment where blood samples were done by erythrocyte cell thinning using 15 minutes incubation then measured hemoglobin in 30 respondents with an average without distinguishing the sex of 12,778 g/dl ± 1,8112. When viewed from the average Hb level of D-IV Health Analyst Students at UNUSA, it was obtained in the normal Hb level category. The results of hemoglobin levels are in accordance with the theory of Nugraha [(12)], that the provisions of the normal price of Hb in men are 13,5-17 g/dl and women 12-15 g/dl.

The results of the primary data in this study obtained an average level of the hemolysis percentage of 1,2%. These results can affect the level of sample thinness, because the smaller the percentage rate of slippage in the blood sample, the lower the hemoglobin level or low. Then vice versa, the greater the percentage value of the level of orality in the blood sample, the more it will affect and experience differences in the more or higher hemoglobin levels. If the level of lysis blood sample is higher, the erythrocytes will be damaged, and so will the more heme and globin that are outside the erythrocytes [(8)].

According to the Veterinary Journal from previous researcher Siswanto, et al.,[(13)], initial hemolysis was characterized by reddish color in the upper layers of plasma and whereas for total hemolysis it was characterized by transparent red without erythrocyte deposits. The presence of these erythrocyte deposits shows that total hemolysis has not yet occurred.

The rate of hemolysis is described as a percent of free hemoglobin in relation to total hemoglobin. Acceptable levels of hemolysis have not been established in North America, but a value of 1% is currently used to assess the biocompatibility of blood storage materials, while the European Council has set a standard of 0,8% [(14)].

The analysis of the results in this study used the One-Way Anova statistical test which showed a significant value of 0,097. So the conclusion drawn Ho accepted that means that there is no significant difference in the results of hemoglobin examination in normal erythrocytes, lysis without incubation and with 15 minutes incubation in the blood.

The results of this study are not in line with the research of Dewi & Durachim [(8)] which suggested that there was a difference in treatment between normal blood samples and lysis blood samples for examination of hemoglobin in the blood. Where disorders of analytic hemolysis occur when plasma constituents are at lower concentrations than constituents in erythrocytes. Release of erythrocytic constituents can result in increased plasma values. While another study from Faruq researchers [(4)], also suggested that there were differences in hemoglobin levels between lysis blood and normal blood by using additional ingredients for lubrication. Whereas in this study it is also in line with research from Ariyadi & Sukeksi [(15)], entitled Differences in the results of direct and indirect Cyanmeth hemoglobin level examination on erythrocytes lysis, that the two methods have no significant or similar differences in hemoglobin results in blood. When viewed from the actual principle the two methods are the same, only difference for indirect Hb examination in erythrocyte lysis is on the way the sample is treated and the length of incubation.

Based on the explanation of the three theories, the researcher analyzed that from the sample of respondents in D-IV Student Health Analysts after treatment in each sample the average percentage of hemolysis was low and most had normal hemoglobin levels. So that conclusions can be drawn according to Hardisari & Koiriyah [(5)], that the smaller the percentage of lysis in red blood samples, the cell wall of erythrocytes does not occur and there is no decrease in hemoglobin levels in the blood. The level of the percentage of lysis that is low or not too high also does not cause a person to suffer from anemia.

Factors that affect hemoglobin and red blood cell (erythrocyte) levels in a person are food, age, sex, activity, smoking and accompanying diseases such as leukemia, thalassemia and tuberculosis. Things that must be considered in the determination of hemoglobin levels include electrical voltage that affects read absorption, the presence of air bubbles, types of anticoagulants, blood clots, homogenization of samples, handling samples, drabkins reagents exposed to light, broken

spectrophotometers and appropriate and appropriate methods to handle Blood samples will be tested can affect the results [(15)].

4. Conclusion

Based on the results and discussion on this research can be concluded that there is no difference between the hemoglobin test results normal sample and hemolysis in the blood of the routine examination used.

5. Thank You Note

Acknowledgments I told Staff Laboratory of Nahdlatul Ulama Surabaya University, Faculty of Health who helped in the research process and to the respondents who participated in the study so that the research can proceed smoothly.

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