

Study of Hemoglobin Levels on Hemolysis Sample

Noor Febryani¹, Ikke Nanda Amalia², Intan Dwi Anggraeni³, Gilang Nugraha³

¹Science of Biological Resources,
The United Graduate School of
Agriculture Science, Gifu
University, Japan

²Department of Basic Medical
Science, Faculty of Medicine,
Airlangga University, Indonesia

³Department of Medical Laboratory
Technology, Faculty of Health,
Nahdlatul Ulama University of
Surabaya, Indonesia

Correspondence:
Gilang Nugraha, Jl. Jemursari No.
51-57, Surabaya, East Java,
Indonesia
Zip Code : 60237

Email: gilang@unusa.ac.id

Received: September 11, 2019
Revised: October 4, 2019
Accepted: October 18, 2019



Abstract

Hemolysis can significantly affect the reliability of test results and occur in the pre-analytical phase. The aim of this study is to reveals the correlation of hemoglobin levels on hemolysis sample. This experimental study was carried out using samples from thirty students of Medical Laboratory Technology study program of the Nahdlatul Ulama University of Surabaya. Blood samples were treated by hard shaken in 30 times in order to damage the middle part of the blood sample. Data on hemoglobin levels were collected and analyzed using the Pearson Correlation Test before and after treatment with significant value $p < 0.05$ indicating that there was a significant correlation. Hemoglobin concentrations were strongly positive for the hemolysis of the sample ($p = 0.000$). The conclusion is that sample hemolysis has a potency to be the confounding factor on the hemoglobin test.

Keywords

Hemoglobin, sample hemolysis, pre-analytical phase

INTRODUCTION

Hematology routine examination is a general hematological examination which are frequently requested in laboratory because it is usually used as an early detection in providing a diagnosis of disease or

monitoring condition of patients. Hemoglobin examination is one of hematological routine examinations which is often in laboratory. There are four principal techniques for measuring hemoglobin, which are tallquist, sulfate of copper, Sahli and

Cyanmethemoglobin (1). The Cyanmethemoglobin technique uses a spectrophotometer or photometer to measure colorimetrically. Cyanmethemoglobin technique has been recommended in hemoglobin examination because that is a reference method, all of types of the hemoglobin can be measured except sulfhemoglobin (8). This technique has been recommended in hemoglobin examination since the error only reaches around 2% (1). Although the Cyanmethemoglobin technique is a reference method, errors that might occur in this examination can be caused by pre-analytical, analytical and post analytical phase. Error which is occur in the pre-analytical phase gives the contribution of 61% of the total laboratory error (9). The errors in the pre-analytical phase may interfere with the reliability of laboratory resultan (2), this happens when especially taking blood samples are often occur hemolysis.

Hemolysis is the most common cause of pre analytical error. Prevention of medical errors is a goal of health care system. The laboratory errors are due to pre analytical variables that has received a great deal of attention. It has been analyzed that hemolysis of patient specimen may interfere with accurate measurement of analytes. Basically, hemolysis occurs when red blood cells become damaged or destroyed. Red blood cells contain hemoglobin molecules which

are released during hemolysis. Once a whole blood specimen is hemolyzed, the hemoglobin molecules within the red blood cells are released causing the serum or plasma to have a pink to red color (10). One of the causes of hemolysis is *in vitro* hemolysis (3). *In vitro* hemolysis is a major instance of error in laboratory testing as it is mainly caused by improper collection and handling of specimens, such as extended use of venous stasis, blood differentiation by means of intravenous catheters and blood collection (4). Furthermore, *in vitro* hemolysis also affects the quality of analyte and can lead to incorrect interpretation of the obtained resultan (5).

When hemolysis occurs in blood cells due to *in vitro* hemolysis, the erythrocyte cell membrane collapses so that the hemoglobin release will broken off the cell membran (6). The examination in the laboratory indicated that the increasing of hemoglobin values are due to the examination result test which was inaccurate. This study of the hemoglobin examination revealed that the range of value was not far apart or still in normal order. Therefore, the purpose of this study reported here was to more thoroughly investigate the correlation of hemolysis in hemoglobin concentration.

MATERIALS AND METHODS

This study was experimental research which are true experimental with measured

pretest-post test control group design. Sample was measured hemoglobin before and after being treated. The blood examination were performed in Laboratory Nahdlatul Ulama University of Surabaya, Faculty of Health, Medical Laboratory Technologist Study Program. A total of thirty samples were collected from the students of Medical Laboratory Technology Study Program, Faculty of Health, Nahdlatul Ulama University in February – April 2019. Ethical clearance was held by Ethics Commission of Nahdlatul Ulama University of Surabaya which was approved this research. Informed consent was given to patient before taking blood samples followed by anonymity and confidentiality data of patient would be guaranteed by researchers.

Samples of blood were taken as much as 3 ml and divided into two sections of the tube. In the first tube, the hemolysis sample was measured directly as a control. While in the second tube, the hemolysis sample was treated by hard shaking of the center portion of the sample vigorously in thirty times and wounded the middle part of the blood sample, then the sample was centrifuged (write down the merek/type of machine) at 4000 rpm for 10 minutes. After the plasma was lysis visible then the hemoglobin levels and percentage of hemolysis were measured. Hemoglobin levels were measured using spectrophotometer Genesys 10s UV-Vis with 540nm wavelength, while on the percentage

of hemolysis was calculated as two measurements. The first measurement of hemolysis in another tube was measured percentage of hemolysis by NaCl 0.1% solution (A_1) and the second measurement of hemolysis was measured percentage of hemolysis by NaCl 0.9% solution (A_2) then both were measured of the absorbance with spectrophotometer Genesys 10s UV-Vis in 540 nm wavelength. The percentage of hemolysis was calculated by using:

$$\% \text{Hemolysis} = \frac{\text{Abs. NaCl 0,9\% solution}}{\text{Abs. NaCl 0,1\% solution}} \times 100\%$$

*Abs: Absorbance

The data of hemoglobin levels before and after treatment were collected and analyzed using Pearson Correlation Test with SPSS version 16.0 to determine the correlation of hemoglobin levels on hemolysis sample with the significance value $p < 0.05$ showed in statistically, there was a significant correlation.

RESULTS

Percentage of Sample Hemolysis

The research were conducted from 30 respondent which were active students of Medical Laboratory Technologist study program, Faculty of Health, Nahdlatul Ulama University of Surabaya. There were consist of men and women student which especially first was measured the percentage of hemolysis, obtained data as follows:

Table 1. Percentage of Hemolysis

Hemolysis (%)	Total
0.7	1
0.8	3
0.9	7
1	1
1.1	2
1.2	6
1.3	4
1.9	1
2	1
2.3	1
2.9	1

Characteristics of Hemoglobin Levels in Various Treatments

The sample of blood was taken as much as 3 ml, subsequently divided into two parts. In the first tube, the hemolysis sample was measured directly as a control. Whereas the hemolysis sample in the second tube was treated by hard shaking the center portion of the sample vigorously in thirty times and wounded the middle part of the blood sample, then the sample was centrifuged at 4000 rpm for 10 minutes in room temperature (20-25°C). After the plasma were lysis visible then the hemoglobin levels were measured. This treated sample is called by Hemolysis

Sample. Characteristics of the samples are summarized in Table 2.

Table 2. Characteristics of hemoglobin level

Parameter	Mean	SD	Range
Control	13.1	1.7	9.7-16.4
Hemolysis Sample	13.8	1.8	10.4-17.2

Correlation of Sample Hemolysis on Hemoglobin Levels

Based on the statistical analysis of the paired samples T-test, the results of the significance value were 0.000. It is indicated that the levels of hemoglobin in the hemolysis sample were different.

Correlation of hemoglobin levels on hemolysis sample using the Pearson Correlation test determined the strength of the correlation of hemoglobin levels on sample hemolysis¹¹. It showed that the significance value was 0.000 and it could be concluded that there was a strong positive correlation of hemoglobin levels on hemolysis sample. These results are shown in the following scatter diagram.

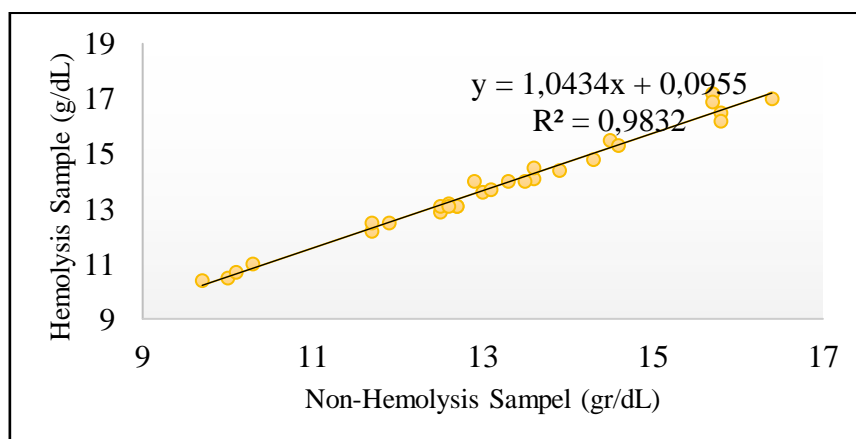


Fig 1. The Scatter Diagram of Hemoglobin Levels

DISCUSSION

Hemolysis is caused by the release of hemoglobin and internal components of erythrocyte membranes into the plasma. Generally, hemolysis is caused by biochemical, physical and immunologic mechanisms (2). *In vitro* hemolysis is usually happened due to errors in collecting and handling of specimen². In the laboratory experiment, the prevalence of hemolysis to reject samples in the hemoglobin test is not known yet. However, the CLSI recommended that the lysed samples were not be tested (2).

The result of this study revealed that a comparison of normal sample and hemolysis sample had significant differences from a blood sample of healthy volunteers who were mechanically induced hemolysis performed *in vitro*. Hemoglobin examination with cyanmethemoglobin technique is used the drabkins solution with the composition of potassium ferricyanide which binding heme (ferro) become (ferri) methemoglobin. Cyanide ion were changed the methemoglobin become Cyanmethemoglobin, KH_2PO_4 are set the pH of solution (7.0-7.4) and non ionic detergent is functioning to accelerate the lysis of erythrocyte. Turbidity in blood sample is consequence of leukosytosis, hyperlipidemia and globulin can cause absorbance measurements to false increase (8).

The hemoglobin levels have been shown to increase in hemoglobin tests with the hemolysis sample in this study. This is not same as the fact that when erythrocyte cells are lysis, it can lead to the disappearance of fluidity or fragility the membranous of the erythrocyte which is easily broken that cause the reduction in hemoglobin level (7).

However, statistical analysis on Pearson Correlation test indicated that was a strong positive correlation of hemoglobin levels on hemolysis sample. The result of this study revealed that hemolysis sample has a potency to be the confounding factor on hemoglobin test. Therefore, further research should be carried out to affect hemoglobin levels on hemolysis sample.

CONCLUSIONS

Based on the result of this study, it can be concluded that there was a strong positive correlation of hemoglobin levels on hemolysis sample. The hemolysis sample has a potency to be the confounding factor on hemoglobin test. This is caused by the turbidity of hemolysis sample so that the hemoglobin levels is false increase.

ACKNOWLEDGEMENTS

We would like to thank the laboratory technicians at the Laboratory of Nahdlatul Ulama University of Surabaya for Technical Support.

CONFLICT OF INTEREST

There are no conflicts of interest.

REFERENCES

1. Nugraha G. Laboratory practice guide on basic hematology. Trans Info Media; 2017.
2. Hernaningsih Y, Akualing JS. The effect of hemolysis on plasma prothrombin time and activated partial thromboplastin time test using photo-optical method. *Medicine*. 2017; 96 (38): 1-5.
3. Michael P, Phelan, Edmund Z, Reineks, Jesse, Schold, Frederic, Chamberlin J, Gary, Procop. Pre analytic factors associated with hemolysis in emergency department blood samples. *Arch Pathol Lab Med*. 2018; 142: 229-235.
4. Adiga US. Hemolytic index-a tool to measure hemolysis in vitro. *IOSR Journal of Biotechnology and Biochemistry*. 2016;2(2): 49-52.
5. Multinovic D, Andrijevic I, Licina M. & Andrijevic L. Confidence Level in Venipuncture and Knowledge on Causes of In Vitro Hemolysis Among Healthcare Professionals. *Biochemia Medica*. 2015; 25(3): 401-409.
6. Kazmierczak SC. Accurate results in the clinical laboratory:a guide to error detection and correction. Amsterdam (Netherlands): Elsevier Academic Press; 2019. Chapter 5, Interferences of hemolysis, lipemia and high bilirubin on laboratory tests; 57-65.
7. Saputro DA, Junaidi S. Giving the vitamin C on physical practice maximum and change the hemoglobin levels and erythrocyte count. 2015; 4(3): 32-40.
8. Norsiah W. The Difference of Hemoglobin Levels Use Sianmethemoglobin Method With and Without Centrifugation on Leucositosis. 2015; 1(2): 72-83.
9. Praptomo, AJ. Quality Control Of Medical Laboratory. Deepublish; 2018.
10. Colvin, H. The Breakdown on Hemolyzed Specimens. 2018; 14(1): 1-2.