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Comparison of Hemoglobin Level Measurement Results Using Sodium-Lauryl Sulphate With Oshiro and Mansoor Procedure

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ABSTRACT

The method of measuring Hb levels recommended by ICSH uses the HiCN method. However, it has a high risk due to the toxicity of the reagent. Therefore, the determination of Hb has been developed using the SLS method. According to Oshiro and Mansoor, who both stated that there was no significant difference between SLS and HiCN, this method has two distinct procedures. This study aims to determine the comparison between the measurement results of hemoglobin levels using sodium lauryl sulfate with the Oshiro procedure and Mansoor. The research design used is analytical research. The data obtained from the results of measuring Hb levels with the Oshiro and Manshoor procedures was given to students of the Diploma IV Study Program of Medical Laboratory Technology, Rajawali Health Institute batch 2018, which collected 49 people. The sampling technique used was saturated sampling to reduce the error rate in the study. The results of the normality test of the data in this study showed n of the two procedures was 49 mean±SD for the Oshiro procedure 13.09±0.56 and p = 0.200. Meanwhile, the Mansoor procedure had a meanSD of 13.09±0.57 and a p-value of 0.059. Because the probability of both being p > 0.05, the data is declared normally distributed. The results of the average difference test from the data of this study show the mean±SD of the pairwise difference between the Oshiro and Mansoor procedures is 0.002±0.059 and the p value = 0.811. If P > 0.05, then it is stated that there is no significant difference between the two groups. The conclusion of this study is that the result of measuring Hb levels using the SLS procedure is reliable. Oshiro and Mansoor conform to HiCN and there is no significant difference in the mean results.

KEYWORDS

Hemoglobin; Cyanmethmoglobin; Sodium Lauryl Sulfate; Oshiro Procedure; Mansoor Procedure

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Introduction

Hemoglobin (Hb) is a complex protein that is in red blood cells and binds to iron (Fe). In the human body, Hb serves to carry oxygen from the lungs to all parts of the body (Nugraha, 2017). High and low Hb levels describe the physical condition of a person, which is influenced by various factors such as age and gender (Faiqah et al., 2018).

The method of measuring Hb levels recommended by the International Committee for Standardization in Haematology (ICSH) is the cyanmethemoglobin method. This method has a high risk for the person conducting the examination because the potassium cyanide and ferricyanide contained in the reagent can be quickly absorbed by the body after inhalation or oral exposure (ATSDR, 2006). Measurement of Hb levels has developed, where measurements are carried out using a non-cyanide method, namely sodium lauryl sulphate (SLS), a surfactant that dissolves cell membrane lipoproteins from red blood cells and then releases Hb and converts it into SLS-Hb (Chaudhray et al., 2017).

The method of measuring Hb levels with sodium lauryl sulfate has several different procedures. According to Oshiro et al. (1982), a measurement of Hb levels requires a concentration of sodium lauryl sulfate in the range of 1.38 to 3.50 mmol/L and the optimal concentration is 2.08 mmol/L. with a measurement procedure of 20 µl of venous blood sample mixed with 5 ml of sodium lauryl sulfate solution (2.08 mmol/L), then readings were taken after 5 minutes on a spectrophotometer with a wavelength of 539 nm. Based on the research results of Oshiro, Toru, and Jiro, they stated that there was no significant difference between the results of measuring Hb levels using sodium lauryl sulfate and cyanmethemoglobin. This method was confirmed by Higgins (2005). Sodium lauryl sulphate (SLS) is a surfactant that lyses erythrocytes and rapidly forms a complex with the released hemoglobin. The concentration of sodium lauryl sulfate solution used was 2.08 mmol/L. The SLS-MetHb product is stable for several hours and has a characteristic spectrum with a maximum absorbance of 539 nm. The complex complies with the Beer-Lambert law so that there is a precise linear correlation between the Hb concentration and the SLS-MetHb absorbance.

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According to Mansoor et al. (2005), the concentration of sodium lauryl sulphate solution of 6.9 mmol/L can be used in the measurement of Hb levels with the procedure of 20 µl of venous blood sample mixed with 3 ml of sodium lauryl sulphate solution. After 20 seconds, readings are taken on a spectrophotometer with a wavelength of 535 nm.The method stated that there was no significant difference between the results of measuring Hb levels using sodium lauryl sulfate and cyanmethemoglobin. The measurement of Hb levels used by Mansoor et al. (2005) refers to research by Lewis et al. (1991). The absorbance readings of Hb levels on a spectrophotometer were carried out at a wavelength of 535 nm, and no significant difference was seen. Based on the absorbance of repeated measurements of Hb levels during the first 4 hours after dilution (mean 0.3488, SD 0.0029, CV 0.83%).

Based on the two procedures, there was no significant difference between the results of measuring Hb levels using Sodium Lauryl Sulfate and Cyanmethemoglobin. The purpose of this study was to compare the results of measuring hemoglobin levels using sodium lauryl sulfate with the Oshiro and Mansoor procedures.

Literature review

Hemoglobin

Red blood cells are a suspension of cells and cytoplasmic fragments in the fluid. Blood functions to transport oxygen needed by cells throughout the body and supplies body tissues with nutrients and metabolic wastes. It also contains various components of the immune system. Each cell morphology has a size (diameter). Blood consists of blood cells and plasma. Blood cells consist of hemoglobin, erythrocytes, hematocrit (PCV), reticulocytes, blood sedimentation rate, platelets, leukocytes, and other types of counts from peripheral blood smear preparations. Hemoglobin stands for the words "haem" and "globin", where haem is Fe and protoporphyrin is mitochondria. Globulin is a chain of amino acids (1 pair chain and 1 pair non). Hemoglobin is composed of iron-containing globular proteins. It is made up of 4 polypeptide chains (acid chain amino acids), i.e., 2 alpha chains and 2 beta chains (Anamisa, 2015). Heme synthesis begins with the condensation of glycine and succinyl-CoA to form amino-aminolevulinic acid (ALA). Then ALA left the mitochondria and formed porphobilinogen. This molecule then returns to the mitochondria and produces protoporphyrin products. Hereinafter, referred to as protoporphyrin IX, which is the final product in the synthesis of heme molecules. To form a complete heme molecule, iron will combine with protoporphyrin. Then the heme will come out of the mitochondria and join the globin molecules that are synthesized on the ribosomes (Parwati, 2018). This synthesis process is indicated by a change in the color of the cytoplasm from dark blue to purple. Before the erythrocyte nucleus disappears, 65% of hemoglobin is synthesized, and 35% on reticulocytes (Kiswari, 2014).Measurement of Hb levels is done to diagnose blood deficiency. A decreased Hb level from normal, means a lack of blood. Adult women should have a normal value of 12–14 g/dl, while adult men should have a value of 14–16 g/dl (Anamisa, 2015).

Cyanmethemoglobin

The method of measuring Hb levels recommended by the International Committee for Standardization in Haematology (ICSH) is cyanmethemoglobin. The cyanmethemoglobin method has advantages, including the availability of stable and internationally accepted references, standards, and calibrators. However, their use can cause problems due to the large volume of disposal reagents containing cyanide, which may be potentially hazardous. As an alternative, the non-toxic sodium lauryl sulfate method has been used. The results of the examination are the same as the HiCN method for measuring hemoglobin at all concentrations. Therefore, this method can be used in primary health care centers, teaching hospitals, and in the diagnostic laboratory (Srivastava et al., 2017).

Sodium Lauryl Sulfate

Measurement of Hb levels has developed where the measurement is carried out by the non-cyanide method, namely sodium lauryl sulfate (SLS), a surfactant that dissolves cell membrane lipoproteins from red blood cells, releasing Hb and converting it to SLS-Hb (Chaudhary et al., 2017). A surface-active agent, or surfactant, is a substance that, when dissolved in a solvent, the molecules of the substance will be attracted to the surface, and its presence can lower the surface tension (Utami, 2008). Besides, In addition, it is also an amphilic molecule that has a hydrophobic group (insoluble in water) and a hydrophilic group (soluble in water). The surfactant head can be 14 zwitterion, anionic, non-ionic, cationic while the tail is a chain non-polar hydrocarbons (Azarmi et al., 2015). Sodium lauryl sulfate (SLS) with the chemical formula $C_{12}H_{25}SO_4Na$ is an anionic surfactant that can be used in the measurement of Hb levels and is a non-toxic chemical substance compared to the cyanmethemoglobin method (Oshiro et al., 1982). Anionic surfactants are surfactants that have a negative charge in the hydrophilic part (Sekhon, 2013).

Oshiro Procedure

Sodium lauryl sulfate is a new method for hemoglobin determination. Oshiro's method of measuring hemoglobin levels requires a concentration of sodium lauryl sulfate in the range of 1.38 to 3.50 mmol/L and the optimal concentration is 2.08 mmol/L, with a measurement procedure of twenty µl of blood being mixed

with 5 ml of the working solution sodium lauryl sulfate and gently shaken, then readings are taken after 5 minutes on a spectrophotometer with a wavelength of 539 nm. Test results are well correlated with the HiCN method (Oshiro et al., 1982).

Mansoor Procedure

The method of measuring Hb levels with sodium lauryl sulfate between Oshiro and Mansoor has different procedures. Twenty µl of blood with EDTA was added into 3 ml of SLS reagent, and the absorbance was read within 20 seconds on a spectrophotometer with a wavelength of 535 nm. A comparison of measurements of methemoglobon by the SLS-Hb Mansoor method and the HiCN method confirmed there was no significant difference (Mansoor, 2005).

Methods

The design of this research is analytic, with the aim of describing or describing the facts of the things being studied systematically and accurately (Husna et al., 2017). This type of research uses a descriptive approach, and the data obtained is quantitative data.

Participants

The population in this study was all students of the 2018 Medical Laboratory Technology DIV Study Program at the Rajawali Bandung Health Institute, totaling 49 people who had agreed between students and researchers in accordance with the code of ethics. Sampling used a saturated sampling technique, so the samples for this study were all students of the 2018 Medical Laboratory Technology Study Program, Rajawali Bandung Institute of Health, class 2018, with the following inclusion criteria: active students of the Rajawali Health Faculty of Health, age range 21–23 years, good body and physically healthy. Exclusion criteria included: no history of anemia or hemoglobin abnormalities; no history of hypertension or hypotension; and no current drug use. The independent variable in this study was the Oshiro and Mansoor procedure, and the dependent variable was the hemoglobin level. The independent variable in this study is the procedure of Oshiro and Mansoor, and the dependent variable is the level of hemoglobin.

Data collection

The data were collected using an observation technique, which means that the primary data was obtained by the researcher conducting a direct examination of hemoglobin. The measurement of hemoglobin levels in this study was first carried out using the Oshiro procedure and followed by the Mansoor procedure. The first step that needs to be taken in determining the hemoglobin level is to make a calibration curve with the aim of using the equation obtained from the calibration curve to determine the hemoglobin level in the sample. The process of making the calibration curve is carried out according to the procedure by Nugraha (2017). The Oshiro procedure uses 5 ml of SLS solution with an optimum concentration of 2.08 mmol/L mixed with 20 µl of venous blood sample. Readings are taken on a spectrophotometer with a wavelength of 539 nm after incubation for 5 minutes. Measurements with the Mansoor procedure used SLS solution with a concentration of 6.9 mmol/L, as much as 3 ml, mixed with 20 µl of venous blood samples. Readings were taken after incubation for 20 seconds with a spectrophotometer at a wavelength of 535 nm. Then each solution that has been reacted to is measured at the appropriate wavelength according to the procedure. The calibration curve was made with the abscissa (x-axis) being the concentration of hemoglobin levels and the ordinate (y-axis) as the absorbance standard. Determination of the hemoglobin content of the sample is done by plotting the absorbance standard on the curve. The sample used in making the calibration curve can be in the form of a control material or a sample with known levels. This study seeks to streamline the costs incurred so that a sample with known hemoglobin levels is used. The sample comes from the results of an examination of hemoglobin levels at a hospital.

Data analysis

The data analysis consisted of a normality test to see whether the data from the measurement of Hb levels was normally distributed or not. If the data is normally distributed, then proceed with the T test to see the difference between the Oshiro and Mansoor measurement results. If the data is not normally distributed, the statistical calculation will use the Wilcoxon test. The results of data processing and analysis using SPSS are in the form of tables and graphs.

Results and Discussion

Calibration Curve Equation

Measurement of hemoglobin levels was carried out by 2 different procedures, namely the procedure according to Oshiro (1982) and the procedure according to Mansoor (2005). The measurement was carried out using a Thermo

Scientific Genesys 10S UV-Vis Spectrophotometer, which then obtained the absorbance value. The absorbance of the measurement results by the device is then calculated using the equation obtained from the calibration curve to determine the hemoglobin level in the sample. The process of making the calibration curve was carried out according to the procedure by Nugraha (2017), in which a standard solution was diluted with an SLS solution (2.08 mmol/L) for the Oshiro procedure and an SLS solution (6.9 mmol/L) for the Mansoor procedure. Then each solution that has been reacted to is measured at the appropriate wavelength according to the procedure. The calibration curve was made with the abscissa (x-axis) being the concentration of hemoglobin levels and the ordinate (y-axis) being the absorbance standard. Determination of the hemoglobin content of the sample is done by plotting the absorbance standard on the curve. The results of making the calibration curve are obtained by the following equation:

Table 1. Calibration Curve Equation

Method/Procedure	Calibration Curve Equation
Oshiro	y = 0,027x + 0,0013
Mansoor	y = 0,027x + 0,0014

Normality Test

The normality test of the data uses the Kolmogorov-Smirnov test, where if the p value > 0.05, then the data is normally distributed or the assumption of normality is met. In Table 2, it can be seen that the results of measuring hemoglobin levels in the Oshiro procedure with as many as 49 samples, have a mean ± SD value of 13.09 ± 0.56 g/dl, a minimum value of 11.8 g/dl, and a maximum value of 14.3 g/dl, and a p value = 0.200. In the Mansoor n column, 49 samples have a mean ± SD value of 13.09 ± 0.57 g/dl, a minimum value of 11.7 g/dl, a maximum value of 14.4 g/dl, and a p value of 0.059. Because the probability value between the Oshiro and Mansoor procedures is 0.200 and 0.059, namely p > 0.05, it is stated that the data is normally distributed. Furthermore, to find out the difference in the average of the two measurement results, a T-test was carried out.

Table 2. Normality Test Results of Hemoglobin Level Measurement Data Using SLS with the Oshiro and Mansoor Procedure

		Mean (g/dl)	SD (g/dl)	Min (g/dl)	Maks (g/dl)	P
Procedure	N					
Oshiro	49	13,09	0,56	11,8	14,3	0,200
Mansoor	49	13,09	0,57	11,7	14,4	0,059

Paired Sample T-Test

In this study, the Paired Sample T-Test method was used because it was to determine the difference in the mean of the two pairs of sample groups. In Table 3, it can be seen that the number of samples, or n, used in both the Oshiro and Mansoor procedures is 49 samples. The results of the difference test (paired sample T-Test) from Oshiro's Hb levels to Mansoor's Hb levels obtained a mean value of ±SD 0.002±0.059 g/dl, and a p value of 0.811. Based on these results, the p-value of the t-test was obtained at 0.811. So, p > 0.05, where the results stated that there was no significant difference from the average comparison of the two procedures.

Table 3. Different Test Results of Hemoglobin Level Measurement Data Using SLS with the Oshiro and Mansoor Procedure

	n	Mean (g/dl)	SD (g/dl)	P
Oshiro's Hb levels and Mansoor's Hb levels	49	0,002	0,059	0,811

Discussion

Calibration Curve Equation

The discussion section shows how the author interprets the results in light of what was already known and tries to explain the new understanding of the problem after taking the results into consideration. The discussion must connect with the introduction, so it explains how your study contributes to the body of knowledge and society. The results of measuring hemoglobin levels using the Oshiro and Mansoor procedures were obtained in accordance with the ICSH recommended procedure. The results obtained are comparable to the results of measurements carried out by the HiCN procedure because SLS is a surfactant that lyses cell membrane lipoproteins from red blood cells, which then releases hemoglobin and converts it into SLS-Hb (Chaudray et al., 2017).

Sodium Lauryl Sulphate (SLS), with the chemical formula C12H25SO4Na, is an anionic surfactant that can be used to measure hemoglobin levels (Oshiro et al., 1982). Anionic surfactants are surfactants that have a negative charge in the hydrophilic part (Sekhon, 2013). The reaction stage in these measurements is after the red blood cells undergo lysis. The absorption of SLS by the red blood cell membrane results in a change in the protein structure. There is a conformational change in the globin molecule. So there is a change in hemoglobin from Fe^{2+} to Fe^{3+}. Methemoglobin (Fe^{3+}) binds to the hydrophilic group of SLS to form a stable complex with a purple color (Siska, 2019).

The mauve color that is formed from these three procedures is due to the strong red color contained in the hem. In human blood, the red color is formed due to a complex bond between heme in the form of ferrous (Fe^{2+}) and oxygen (O_2) to form a bright red color (Lutz, 2010). In a measurement, there is a reaction between heme in the form of ferrous (Fe2+), which is then oxidized to ferric (Fe^{3+}) or methemoglobin to form a stable red color. Methemoglobin is a derivative of hemoglobin where Fe^{2+} is oxidized to Fe^{3+}, which results in the inability of hemoglobin to bind oxygen (O_2) reversibly, while the polypeptide chain is not changed (Kiswari, 2014). Thus, the color formed from the reaction of the three procedures is mauve.

Based on the results of the study, to determine a significant comparison of results, the normality test of the data was carried out first. The normality test of this data was conducted to determine whether the measurement results of hemoglobin levels were normally distributed or not. If so, these results could be used as a basis for determining the method used in the difference test. The normal distribution is a form of probability distribution that uses a normal curve approach, wherein in the normal curve the distribution is considered even. The method for testing the normality of the research data uses the Kolmogorov-Smirnov test, where the decision-making principle of this result is that if the p value > 0.05, the data can be declared normally distributed (Dahlan, 2014).

The results of the data normality test can be seen that the results of measuring hemoglobin levels in the Oshiro and Mansoor procedures with n as many as 49 samples have an overall mean±SD value of 13.09±0.56 and both procedures have p values of 0.200 and 0.059. So, the data is declared normally distributed. Based on the data, it is known that the data is normally distributed. The difference test is then carried out with the T test. The test model used is the Paired Sample T-Test. It is used because it is used to determine the difference in the average of the two paired sample groups (Dahlan, 2014).

Based on the results of the study, it is known that the number of samples, or n used in the Oshiro and Mansoor procedures is 49 samples. The mean±SD of the pairwise difference between the Oshiro and Mansoor procedures was 0.002±0.059 and the p value=0.811. Based on these results, the p-value of the t-test was obtained at 0.811. So, p > 0.05, where the results stated that there was no significant difference from the average comparison of the two procedures.

The results of this study are in accordance with previous research conducted by Oshiro et al. (1982), as well as Mansoor et al. (2005), where the two procedures did not show significantly different results between the results of measurements using the SLS method and the SLS method recommended by ICSH. Both procedures have met the Lambert-Beer law criteria whereby the maximum wavelength is used in accordance with the characteristics of the spectrum and the concentration of a sample.

Conclusion

The results of the study "Comparison of Hemoglobin Level Measurement Results Using Sodium Lauryl Sulphate With Oshiro and Mansoor Procedure" showed that there was no significant difference between the results of the comparison of the results of measuring hemoglobin levels using SLS with the Oshiro and Mansoor procedures. Thus, it can be concluded that the Cyanmethemoglobin method of hemoglobin examination can be replaced by using a safer SLS reagent, namely the Oshiro or Mansoor procedures.

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