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Differences In Hemoglobin Levels In Whole Blood Before and After 14 Days Of Storage

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Abstrak

Whole blood (WB), yang juga dikenal sebagai darah lengkap, merupakan produk darah yang mengandung seluruh komponen darah, seperti trombosit, eritrosit, leukosit, dan plasma. Penggunaan whole blood hanya dibenarkan apabila diperlukan peningkatan kapasitas pengangkutan oksigen sekaligus penambahan volume darah, seperti pada kondisi perdarahan akut, syok hipovolemik, dan trauma berat dengan perdarahan lebih dari 1.500 ml. Penelitian ini bertujuan untuk mengetahui apakah terdapat perbedaan kadar hemoglobin pada whole blood setelah masa penyimpanan selama 14 hari di Unit Donor Darah (UDD) Palang Merah Indonesia (PMI) Kabupaten Serang. Penelitian ini menggunakan jenis penelitian analitik observasional dengan pendekatan kuantitatif. Populasi penelitian berjumlah 40 sampel dan pengambilan sampel dilakukan menggunakan teknik total sampling, sehingga jumlah sampel yang digunakan sebanyak 40 sampel. Data kemudian diolah menggunakan Microsoft Excel dan IBM SPSS versi 26. Hasil penelitian disajikan dalam bentuk tabel distribusi frekuensi, grafik, dan persentase. Hasil perhitungan uji-t menunjukkan nilai p = 0,007. Karena nilai tersebut lebih kecil dari 0,05, dapat disimpulkan bahwa terdapat perbedaan yang signifikan pada kadar hemoglobin whole blood sebelum penyimpanan (hari ke-1) dan setelah penyimpanan (hari ke-14). Dengan demikian, komponen whole blood (WB) di UDD PMI Kabupaten Serang menunjukkan adanya perbedaan kadar hemoglobin antara sebelum dan setelah penyimpanan selama 14 hari.

Kata Kunci: *Darah Lengkap (Whole Blood); Kadar Hemoglobin; Masa Penyimpanan 14 Hari.*

Abstract

Whole blood (WB), also known as complete blood, is a complete product of blood that contains all its components, such as platelets, erythrocytes, leukocytes, and plasma. The use of whole blood is justified only when the oxygen-carrying capacity and volume expansion required are provided for conditions such as acute bleeding, hypovolemic shock, and major trauma with bleeding of more than 1500 ml. The goal is to find out if whole blood at the Indonesian Red Cross Serang Regency Blood Donation Unit (BDU) has different haemoglobin levels after a 14-day storage period. Using total sampling with a sample size of 40, this study employs an observational analytical type with a quantitative approach and a population of 40. Microsoft Excel and IBM SPSS version 26 were then used to process the data, and the outcomes are displayed as frequency distribution tables, graphs, and percentages. The T-test computation produced a P value of 0.007. The haemoglobin levels in whole blood before storage (day 1) and after storage (day 14) differ significantly, as indicated by the value being less than 0.05. The whole blood (WB) components at the BDU Indonesian Red Cross Serang Regency had different haemoglobin levels before and after 14 days of storage.

Keywords**:** *Whole Blood; Hemoglobin Levels; 14-day Storage Period.*

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INTRODUCTION

Blood transfusion is an essential component of health care and is primarily intended to support the treatment and recovery of patients rather than to generate personal or commercial benefits. Blood is a public health resource and, therefore, should not be traded or commercialized under any circumstances. The provision of safe, adequate, accessible, and affordable blood and blood components is consequently an important responsibility of the health system. In Indonesia, the government regulates blood transfusion services to ensure that transfusion practices are conducted according to established safety, quality, and community health requirements (Permenkes, 2015). The adequacy of the national blood supply remains an important challenge. With a population of approximately 255 million people, Indonesia was estimated to require approximately 5.2 million blood units annually based on the World Health Organization (WHO) recommendation, whereas the available supply was reported to be approximately 4.2 million units, leaving an estimated deficit of nearly one million units (Nurhidayatullah, 2019). This gap highlights the importance of maintaining not only an adequate quantity of donated blood but also its quality throughout collection, processing, storage, and transfusion.

One of the important parameters used to assess the quality and suitability of donated blood is hemoglobin (Hb) concentration. Hemoglobin is an iron-containing protein located within erythrocytes and plays a central role in transporting oxygen from the lungs to body tissues and facilitating the transport of carbon dioxide in the opposite direction. Consequently, an adequate Hb concentration is essential for ensuring the oxygen-carrying capacity of blood used for transfusion. International and national blood-transfusion standards therefore require appropriate donor screening, including assessment of Hb concentration before blood collection. A donor with an insufficient Hb concentration may contribute blood with a reduced oxygen-carrying capacity, while the Hb concentration of collected blood may also change during subsequent storage. Monitoring Hb concentration is consequently important not only for protecting donors but also for ensuring that blood products meet therapeutic requirements and remain suitable for clinical use. Whole blood collected and stored by blood donation units must comply with established quality standards to ensure patient safety and transfusion effectiveness.

Whole blood (WB) refers to blood collected into a sterile blood bag containing an approved anticoagulant solution and includes the major cellular and plasma components, namely erythrocytes, leukocytes, platelets, and plasma. A standard unit of whole blood generally contains approximately 450 mL of blood together with 63 mL of anticoagulant, whereas blood collection practices in Indonesia may involve units containing 250 mL of blood with 37 mL of anticoagulant or 350 mL with 49 mL of anticoagulant (Dipayanaya, 2020). Whole blood may be transfused without further processing when clinically indicated, although it can also be processed into specific blood components, including packed red cells (PRC), platelet concentrates (TC), and fresh frozen plasma (FFP). In contemporary transfusion practice, component therapy is generally preferred because it enables the specific blood component required by a patient to be administered while minimizing unnecessary exposure to other components. Blood components can be prepared from whole blood through centrifugation or obtained by apheresis, in which the required component is collected from the donor while the remaining components are returned to the donor.

Based on storage duration, whole blood can broadly be distinguished according to the extent to which cellular and coagulation components are preserved. Fresh whole blood stored for a relatively short period retains a greater proportion of functional platelets and coagulation factors, whereas prolonged storage is associated with progressive depletion or alteration of several labile components. During storage, metabolic and biochemical changes occur within blood cells, resulting in the accumulation of metabolites such as lactate, ammonia, and potassium and alterations in other biochemical parameters (Permenkes, 2015). These changes are part of the so-called storage lesion, which encompasses morphological alterations, depletion of adenosine triphosphate (ATP), reduction in 2,3-diphosphoglycerate (2,3-DPG), changes in erythrocyte mechanical properties, membrane alterations, and oxidative damage. The extent of these changes is influenced by storage duration, temperature, anticoagulant-preservative systems, and the characteristics of the blood product.

The anticoagulant-preservative solution is an important factor in maintaining the quality of stored blood. Citrate phosphate dextrose adenine (CPDA-1) is widely used for the preservation of whole blood and erythrocytes. Citrate acts as an anticoagulant by binding calcium, phosphate functions primarily as a buffering agent, dextrose provides an energy substrate for erythrocyte metabolism, and adenine supports ATP synthesis in red blood cells. The inclusion of adenine in the CPD solution improves erythrocyte preservation and extends the allowable storage period from approximately 21 days to 35 days. Under appropriate storage conditions, erythrocytes collected with CPDA-1 can therefore be stored for up to 35 days at approximately 2–6°C (Permenkes, 2015). In some preservation systems, additional solutions containing glucose and other substrates can further extend erythrocyte storage to approximately 42 days while maintaining an acceptable hematocrit. Nevertheless, prolonged storage is accompanied by progressive cellular aging and biochemical deterioration, similar in some respects to the aging process of erythrocytes in vivo. Following transfusion, a proportion of stored erythrocytes may consequently be removed more rapidly by the recipient's reticuloendothelial system. Potassium leakage from erythrocytes is another recognized consequence of prolonged storage.

Temperature control is essential throughout blood storage and transportation. Blood and blood components requiring refrigeration should generally be maintained at 2–6°C to minimize metabolic activity, reduce biochemical deterioration, and limit the accumulation of metabolic waste products. Appropriate

temperature maintenance must begin immediately after blood collection and continue during transportation, for which validated cold-storage containers or other suitable temperature-controlled systems should be used. Maintaining the recommended temperature is particularly important because deviations from the established storage range may accelerate biochemical and cellular changes and potentially compromise blood quality. Therefore, storage conditions should be continuously controlled and monitored as part of a comprehensive blood-quality management system.

Prolonged storage may also affect the oxygen-delivery capacity of erythrocytes. During storage, ATP and 2,3-DPG concentrations progressively decline, while metabolic products such as lactate and ammonia may accumulate and extracellular potassium may increase. The reduction in 2,3-DPG increases the affinity of hemoglobin for oxygen, which may temporarily reduce the ability of transfused erythrocytes to release oxygen efficiently to peripheral tissues. Previous research has demonstrated that the concentration of Hb and other biochemical parameters may change during storage, although the magnitude and statistical significance of these changes vary according to the storage period and study conditions. Frideyenti (2022), for example, compared hemoglobin-related characteristics of blood stored for one day and 15 days and reported reductions in ATP and 2,3-DPG during storage. These changes are relevant because ATP contributes to the maintenance of erythrocyte membrane integrity, whereas 2,3-DPG plays an important role in regulating the affinity of hemoglobin for oxygen. Similarly, research conducted by Pesalmen Saragih (2019) examining the effect of packed red cell storage duration on hematocrit, plasma glucose, and hemoglobin concentration found an increase in hemoglobin concentration during seven days of storage, although the observed change was not statistically significant.

The effects of storage are particularly relevant when considering the clinical indications for whole blood transfusion. Whole blood is generally reserved for selected clinical situations, particularly substantial acute blood loss in which both circulating red cell mass and plasma volume need to be restored. In cases of active hemorrhage involving the loss of approximately 25–30% or more of total blood volume, whole blood can simultaneously provide erythrocytes and plasma volume. Nevertheless, the immediate restoration of circulating volume is often a more urgent therapeutic objective than the replacement of erythrocytes alone, and therefore whole blood is not necessarily the first-line transfusion product in every clinical circumstance. Current transfusion practice increasingly emphasizes component-specific therapy, allowing patients to receive PRC, TC, or FFP according to their clinical requirements. This approach also supports more efficient utilization of donated blood by enabling a single whole-blood donation to be separated into several therapeutic components.

The relationship between storage duration and hemoglobin concentration is therefore an important consideration in evaluating the quality of stored whole blood. Although Hb concentration is only one indicator of blood quality, it provides valuable information concerning the erythrocyte content and potential oxygen-carrying capacity of a blood product. Previous findings indicate that storage can produce measurable changes in erythrocyte metabolism and function, particularly after several weeks of refrigeration. However, the presence of storage-related changes does not necessarily mean that a blood product is unsuitable for transfusion, provided that it has been collected, processed, stored, and monitored according to applicable standards. Accordingly, evaluating Hb concentration at different storage periods can contribute to a better understanding of the stability of whole blood during routine storage and can provide empirical evidence regarding whether stored blood remains within the therapeutic quality range.

A preliminary study conducted at the Indonesian Red Cross (PMI) Blood Donation Unit (UDD) in Serang Regency in early 2025 indicated that blood demand could reach approximately 1,000 units per month, while donations were obtained through both fixed-site collection and mobile blood-donation activities. Daily transfusion requirements varied considerably and were not always consistent with the quantity of blood available in the blood bank. This discrepancy between demand and supply emphasizes the importance of ensuring that available blood units can be safely stored and remain therapeutically suitable throughout their permitted storage period. Based on this context, the present study focuses on the changes in hemoglobin concentration in newly collected and stored whole blood and evaluates whether the observed differences remain within the applicable therapeutic quality standards under appropriate storage conditions. The findings are expected to provide practical evidence for blood-bank management, particularly in determining the quality and suitability of stored whole blood for transfusion in situations where immediate use of freshly collected blood is not feasible.

METHOD

Research Design

This study employed an analytical observational design to investigate the association between whole-blood storage duration and hemoglobin concentration. The study was conducted during the first week of March 2025 using whole-blood units stored in a blood bank refrigerator under standardized storage conditions. The study did not involve any intervention or manipulation of the blood samples; instead, hemoglobin concentration was assessed according to the storage duration of each whole-blood unit.

Population and Sample

The study population comprised 40 whole-blood units available during the study period. Given the relatively limited population size and the fact that all available blood units met the predetermined inclusion criteria, a total sampling technique was applied. Consequently, all 40 whole-blood units were included in the study, resulting in a final sample size of 40 units. The independent variable was the storage duration of whole blood, whereas the dependent variable was the hemoglobin concentration of the whole-blood units.

Data Collection and Laboratory Examination

Data were obtained through direct laboratory examination of the whole-blood samples. Each whole-blood unit was stored in a blood bank refrigerator at the recommended storage temperature, and hemoglobin concentration was assessed according to the corresponding storage duration, with observations covering a period of up to 14 days. Prior to laboratory analysis, the samples were assessed for hemolysis to ensure that the specimens met the requirements for examination. Hemoglobin concentration was subsequently measured using a Plasma Low Hb instrument according to the applicable laboratory examination procedures. The results obtained from the laboratory measurements constituted the primary data and were recorded systematically for subsequent statistical analysis.

Data Analysis

The collected data were organized and presented in tabular form and analyzed using descriptive and inferential statistical methods. Univariate analysis was performed to describe the distribution and characteristics of each study variable, including whole-blood storage duration and hemoglobin concentration, using appropriate measures of central tendency and dispersion. Bivariate analysis was subsequently conducted to examine the association between storage duration and hemoglobin concentration. The statistical test was selected according to the scale of measurement and distribution of the data. Prior to inferential analysis, the normality of the data distribution was assessed to determine the appropriate parametric or non-parametric statistical procedure. The statistical analysis was performed to determine whether differences in storage duration were associated with changes in hemoglobin concentration in stored whole blood.

RESULTS AND DISCUSSION

RESULTS

In this study, 40 whole-blood units were obtained from the Blood Donation Unit (UDD) of the Indonesian Red Cross (PMI) in Serang Regency in March 2025. The data collected included blood group, hemoglobin concentration measured on day 1 and day 14 of storage, and the type of anticoagulant used in the blood collection bags. The collected data were processed using IBM SPSS Statistics version 26 and Microsoft Excel 2010 and subsequently presented in tabular form.

a. The characteristics of the samples and the results of the laboratory examinations are described below.

Table 1. Sample Results By Blood Type

No	Blood Type and Rh Factor	Frequency	Percentage
1	A Rhesus pos	12	30 %
2	B Rhesus pos	8	20 %
3	O Rhesus pos	13	32,5%
4	AB Rhesus pos	7	17,5%
	Total	40	100 %

Source: Secondary Data, 2025

Table 1 shows the distribution of the 40 whole-blood samples according to ABO blood group. Blood group O was the most frequently represented, comprising 13 samples (32.5%), followed by blood group A with 12 samples (30.0%), blood group B with 8 samples (20.0%), and blood group AB with 7 samples (17.5%). Thus, blood group O constituted the largest proportion of the study samples, although its distribution was only slightly higher than that of blood group A. This distribution differs from the pattern reported in a previous study by Barot et al. (2020), indicating that the composition of blood groups may vary across study populations and geographic settings. National population data reported by the Directorate General of Population and Civil Registration (Dukcapil) indicate that blood group O is also widely represented among the Indonesian population, with 17,284,056 individuals reportedly classified as blood group O nationwide. In Serang, blood group O was likewise reported to represent a substantial proportion of the population, with the number of individuals belonging to this blood group estimated to reach approximately 1.50 million in 2024.

b. Presentation of data by type of anticoagulant

The following table shows the characteristics of the samples by type of anticoagulant:

Table 2. Frequency Distribution By Type Of Anticoagulant

No	Types of Anticoagulants	Frequency	Percentage
1	CPDA 1	40	100 %
2	CPDA	0	0 %
3	SAGM	0	0 %
Total		40	100 %

Source: Secondary Data, 2025

The distribution of whole-blood samples according to the type of anticoagulant used is presented in Table 2. All 40 whole-blood units (100%) were collected and stored using citrate phosphate dextrose adenine (CPDA-1) as the anticoagulant-preservative solution. Thus, no variation in anticoagulant type was observed among the study samples. The use of a single anticoagulant type across all samples provided a relatively uniform storage condition for the assessment of changes in hemoglobin concentration between day 1 and day 14 of storage.

CPDA-1 is widely used for blood collection and storage because its components support the preservation of erythrocyte viability during refrigerated storage. The solution contains citrate, phosphate, dextrose, and adenine, which serve complementary functions in maintaining the biochemical and metabolic stability of erythrocytes. Citrate acts as an anticoagulant by binding calcium, phosphate contributes to buffering capacity, dextrose provides an energy substrate for erythrocyte metabolism, and adenine supports ATP synthesis. These properties contribute to the maintenance of erythrocyte integrity and prolong the permissible storage period of whole blood under appropriate conditions (Permenkes, 2015).

c. Data Presentation Based on Hemoglobin Levels on Day 1 (Before Storage)

The following table shows the characteristics of the samples based on hemoglobin levels on the first day (before storage):

Table 3. Frequency Distribution Based On Hemoglobin Levels On Day 1 (Before Storage)

No	Hemoglobin Level on Day 1 (Before Storage)	Frequency	Percentage
1	12 gr/dl	2	5 %
2	13 gr/dl	14	35 %
3	14 gr/dl	11	27,5 %
4	15 gr/dl	11	27,5 %
5	16 gr/dl	2	5 %
Total		40	100 %

Source: Secondary Data, 2025

Table 3 presents the distribution of hemoglobin (Hb) concentrations in the whole-blood units on the first day of storage. The Hb concentration varied among the samples, with the highest frequency observed at 13 g/dL, comprising 14 samples (35.0%). The lowest Hb concentrations were 12 g/dL and 16 g/dL, each occurring in 2 samples (5.0%). These findings indicate that the Hb concentrations of the whole-blood units were generally within a relatively narrow range on the first day of storage. The observed pattern is consistent with the findings of Desmawati (2018), who reported that hemoglobin concentrations during the initial stage of storage were generally within the range of approximately 13–15 g/dL. Nevertheless, Hb concentration may change progressively during storage as erythrocytes undergo biochemical and structural alterations, including changes in cellular viability, erythrocyte metabolism, membrane integrity, and the potential development of hemolysis. Such storage-related changes may influence the quality and functional characteristics of erythrocytes and should therefore be considered when evaluating the stability of stored whole blood.

d. Presentation of data based on hemoglobin levels on day 14

The characteristics based on hemoglobin levels on the fourteenth day are shown in the following table:

Table 4. Frequency Distribution Based On Hemoglobin Levels On Day 14

No	Hemoglobin Level on Day 14	Frequency	Percentage
1	12 gr/dl	11	27,5 %
2	13 gr/dl	11	27,5 %
3	14 gr/dl	16	40 %
4	15 gr/dl	2	5 %
5	16 gr/dl	0	0 %

Total	40	100 %

Source: Secondary Data, 2025

The results obtained on the fourteenth day of storage showed a variation in hemoglobin (Hb) concentration among the whole-blood units. The highest observed Hb concentration was 14 g/dL, which was recorded in 16 samples (40.0%). The distribution of the remaining Hb concentrations is presented in Table 4. Compared with the measurements obtained on the first day of storage, the Hb concentration on day 14 demonstrated changes that may be associated with the progressive effects of blood storage. These findings are consistent with Mason (2021), who reported that hemoglobin-related changes during blood storage may be influenced by several factors, including the type of anticoagulant-preservative solution, storage temperature, storage duration, erythrocyte metabolism, the gaseous environment within the storage system, and donor-related characteristics.

During storage, erythrocytes undergo progressive biochemical and structural changes collectively referred to as storage lesions. As storage continues, metabolic by-products such as lactate may accumulate, while alterations in erythrocyte membrane integrity and permeability can affect cellular function and viability. These changes may increase the susceptibility of erythrocytes to membrane damage and hemolysis, potentially resulting in the release of intracellular hemoglobin into the surrounding plasma. Consequently, changes in measurable hemoglobin concentration may occur during prolonged storage. Although CPDA-1 is formulated to preserve erythrocyte viability by providing anticoagulant and metabolic support, the protective capacity of the preservation system is not unlimited and cellular deterioration may progressively occur during storage. In addition, maintaining an appropriate storage temperature of 2–6°C is essential for minimizing metabolic activity and cellular deterioration. Temperature fluctuations or inappropriate storage conditions may accelerate erythrocyte damage and consequently affect the quality of stored blood (Kemenkes, 2015).

The changes observed between the first and fourteenth days of storage should therefore be interpreted in the context of the overall storage environment rather than being attributed solely to the anticoagulant used. Because all blood units in the present study were stored using CPDA-1, the effect of anticoagulant type could not be independently evaluated. Instead, the observed differences in hemoglobin concentration are more appropriately considered in relation to storage duration and the physiological and biochemical changes that occur in erythrocytes during refrigerated storage.

e. Results of the Analysis of Differences in Whole Blood Hemoglobin Levels Before and After 14 Days of Storage at the PMI Blood Donation Unit in Serang Regency

1) Test of data normality

A normality test was conducted to ensure that the residuals in the regression model followed a normal distribution. Sugiyono (2015) states that the normality of research data is determined using the Kolmogorov-Smirnov (K-S) statistical test.

The researcher used an exact Monte Carlo test with a 99% confidence level, which provided the basis for decision-making regarding the normality test.

TABLE 5. Results of the Normality Test

One-Sample Kolmogorov-Smirnov Test

N			40
Normal Parameters[a,b]	Mean		.0000000
	Std. Deviation		1.31264659
Most Extreme Differences	Absolute		.071
	Positive		.049
	Negative		-.071
Test Statistic			.071
Asymp. Sig. (2-tailed)			.200[c,d]
Monte Carlo Sig. (2-tailed) Sig.			.667[e]
	99% Confidence Interval	Lower Bound	.655
		Upper Bound	.679

a. Test distribution is Normal.
b. Calculated from data.
c. Lilliefors Significance Correction.
d. This is a lower bound of the true significance.
e. Based on 10000 sampled tables with starting seed 2000000. Sumber: *IBM SPSS Statistics* 26 (2025)

Source: Primary data, processed in 2025

The Monte Carlo significance value of 0.667 is greater than 0.05 in the normality test using the exact Monte Carlo Kolmogorov-Smirnov test, indicating that the data are normally distributed.

2) Linearity Test

The results of the linearity test comparing hemoglobin levels on the first and fourteenth days are shown below:

Table 6. Linearity Test Results

Group Statistics

	Kode	N	Mean	Std. Deviation	Std. Error Mean
KadarHB	Hari 1	40	13,93	1,023	,162
	Hari 14	40	13,23	,920	,145

Source: Primary data, processed in 2025

Forty samples on the first day showed an average hemoglobin level of 13.93 g/dL with a standard deviation of 1.023, and 40 (forty) samples on the fourteenth day showed an average hemoglobin level of 13.23 g/dL with a standard deviation of 0.920.

Hemoglobin levels for each variable are presented in descriptive statistics. On the first day, the average hemoglobin level was higher than on the fourteenth day, indicating that hemoglobin levels before and after the storage period were not the same.

Table 7. Linearity Test Results

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
KadarHB	Equal variances assumed	,140	,709	3,219	78	,002	,700	,217	,267	1,133
	Equal variances not assumed			3,219	77,137	,002	,700	,217	,267	1,133

Source: Primary data, processed in 2025

The results of the comparison of hemoglobin (Hb) concentrations between whole blood on day 1 and day 14 are presented in Table 7. Levene's test indicated no significant difference in variance between the two groups ($F = 0.140$; $p = 0.709$), indicating that the assumption of equal variances was satisfied. Therefore, the analysis was interpreted using the equal variances assumed row of the independent-samples t-test. The analysis showed a mean difference in Hb concentration of 0.700 g/dL, indicating that the Hb concentration on day 1 was, on average, 0.700 g/dL higher than that on day 14. The 95% confidence interval for the mean difference ranged from 0.267 to 1.133 g/dL, indicating that the true mean difference in Hb concentration between the two groups was estimated to fall within this range. The independent-samples t-test demonstrated a statistically significant difference between the two groups ($t = 3.219$, $df = 78$, $p = 0.002$). Since the p-value was below the significance level of 0.05, the null hypothesis was rejected. These findings indicate that hemoglobin concentration differed significantly between whole-blood samples assessed on day 1 and those assessed on day 14 of storage, with the day-14 samples showing a lower mean hemoglobin concentration.

DISCUSSION

The present study examined the characteristics of whole-blood samples and the changes in hemoglobin (Hb) concentration during storage, with particular attention to the comparison between measurements obtained on day 1 and day 14. The variables examined included ABO blood group, anticoagulant type, storage duration, and hemoglobin concentration. These variables are relevant to transfusion quality because the composition and preservation conditions of blood may influence the stability and functional characteristics of erythrocytes during storage. The findings demonstrated that the distribution of blood groups varied among the samples, all blood units were preserved using CPDA-1, and hemoglobin concentration differed significantly between the first and fourteenth days of storage.

The distribution of ABO blood groups showed that group O was the most frequently represented blood group, accounting for 32.5% of the samples, followed by group A (30.0%), group B (20.0%), and group AB (17.5%). The predominance of blood group O in the present sample is relevant because ABO distribution may differ according to geographic location, population characteristics, and donor composition. The finding is

broadly consistent with population-level information indicating that blood group O is highly prevalent in Indonesia. However, the distribution observed in blood-bank samples should not be interpreted as directly representing the general population because donor recruitment, donation frequency, demographic characteristics, and local transfusion demand may influence the composition of blood stocks. The difference between the present findings and those reported by Barot et al. (2020) further indicates that variation in ABO blood-group distribution may occur across different populations and study settings. Nevertheless, because blood group was not the primary explanatory variable in the present study, its principal role was to describe the composition of the blood samples rather than to explain changes in hemoglobin concentration.

Another important variable was the anticoagulant-preservative solution. All blood units included in this study used citrate phosphate dextrose adenine (CPDA-1), resulting in a uniform anticoagulant composition across the samples. CPDA-1 is designed to maintain erythrocyte viability during refrigerated storage through the complementary functions of citrate, phosphate, dextrose, and adenine. Citrate prevents coagulation through calcium chelation, phosphate contributes to buffering, dextrose provides an energy substrate for erythrocyte metabolism, and adenine supports ATP synthesis. Adequate ATP availability is important for maintaining erythrocyte membrane integrity and cellular deformability during storage (Permenkes, 2015). The use of the same anticoagulant in all samples can therefore be regarded as an advantage because it reduces variation attributable to differences in preservation systems. However, because no alternative anticoagulant was included, the present study cannot determine whether CPDA-1 is superior to other anticoagulant-preservative solutions or whether the type of anticoagulant independently affects Hb concentration.

The principal finding of this study was the difference in Hb concentration between day 1 and day 14 of storage. The mean Hb concentration on day 1 was 0.700 g/dL higher than that observed on day 14. The 95% confidence interval for the mean difference ranged from 0.267 to 1.133 g/dL, indicating that the estimated difference remained above zero across the confidence interval. Statistical analysis confirmed that the difference was significant ($t = 3.219$, $df = 78$, $p = 0.002$). This finding indicates that storage duration was associated with a measurable change in Hb concentration during the 14-day storage period. Importantly, the statistical result demonstrates an association or difference between the two storage periods and should not be interpreted as definitive evidence that storage duration alone caused the change, particularly because several other storage-related factors may contribute to alterations in erythrocyte characteristics.

The reduction in Hb concentration observed after 14 days may be related to progressive biochemical and structural changes occurring in stored erythrocytes. During storage, erythrocytes continue to undergo metabolism despite refrigeration, resulting in the accumulation of metabolic by-products and gradual depletion of intracellular energy substrates. ATP concentration decreases over time, while 2,3-diphosphoglycerate (2,3-DPG) also declines. These alterations may affect erythrocyte membrane stability, deformability, and oxygen-delivery characteristics. Changes in membrane permeability may further contribute to cellular damage and increase the risk of hemolysis, potentially resulting in the release of intracellular hemoglobin into the storage medium. Therefore, the observed difference in Hb concentration between day 1 and day 14 may reflect the cumulative effects of erythrocyte storage lesions.

The present finding is consistent with previous research describing progressive biochemical changes in stored blood. Frideyenti (2022) reported changes in ATP and 2,3-DPG during blood storage, emphasizing the progressive metabolic alterations that occur in erythrocytes after collection. The reduction in ATP is particularly important because ATP contributes to the maintenance of erythrocyte membrane integrity, whereas decreased 2,3-DPG alters the affinity of hemoglobin for oxygen. Similarly, Pesalmen Saragih (2019) investigated the effect of packed red cell storage duration on hematocrit, plasma glucose, and Hb concentration and reported changes in Hb concentration during storage, although the observed increase was not statistically significant over seven days. The difference between that finding and the significant difference observed in the present study may be attributable to differences in storage duration, blood product characteristics, sample composition, study design, and laboratory conditions. Mason (2021) also emphasized that changes in stored blood are influenced by multiple factors, including the anticoagulant-preservative solution, storage temperature, storage duration, erythrocyte metabolism, the storage environment, and donor-related characteristics. Thus, the present findings support the broader evidence that blood storage is accompanied by progressive changes in erythrocyte characteristics.

The clinical significance of these findings lies in the importance of maintaining the quality of stored blood until it is transfused. Hemoglobin concentration is one of the important indicators of erythrocyte content and oxygen-carrying capacity. Although a statistically significant reduction in Hb concentration was observed after 14 days, statistical significance alone does not establish that the stored blood was clinically unsuitable for transfusion. Blood quality must be evaluated using multiple parameters and against established regulatory standards. Consequently, Hb concentration should be interpreted together with other indicators of storage quality, including hematocrit, hemolysis, potassium concentration, ATP, 2,3-DPG, and morphological or membrane changes when such examinations are available.

This study has several strengths. First, the study used blood samples obtained directly from a blood donation unit, providing real-world data under routine blood-bank storage conditions. Second, the use of a uniform CPDA-1 anticoagulant-preservative system reduced variability associated with different

anticoagulants. Third, hemoglobin concentration was evaluated at two defined storage periods, allowing an objective assessment of changes occurring during storage. The use of standardized statistical analysis and reporting of the mean difference, confidence interval, and p-value also strengthens the interpretation of the findings.

Nevertheless, several limitations should be considered. The sample size was relatively small, and the study was conducted at a single blood donation unit in Serang Regency; therefore, the findings may not be generalizable to other blood banks or populations. In addition, the study focused primarily on Hb concentration and did not simultaneously assess other important indicators of storage lesions, such as hemolysis, ATP, 2,3-DPG, hematocrit, or potassium concentration. Because all samples used CPDA-1, the independent effect of anticoagulant type could not be evaluated. Donor-related variables and detailed storage conditions may also contribute to variation in Hb concentration but were not comprehensively analyzed. Future studies should therefore involve larger samples, multiple blood-donation facilities, longer observation periods, and additional biochemical and hematological parameters to provide a more comprehensive assessment of blood-storage quality.

CONCLUSION

The results of a study on changes in whole blood hemoglobin levels before and after fourteen days of storage showed that the whole blood (WB) hemoglobin level on the first day was 13 g/dL (28%) and on the fourteenth day was 14 g/dL (40%). On the first and fourteenth days, there was a general difference in whole blood hemoglobin levels between the first and fourteenth days, with an average difference of 0.70 g/dL.

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