

Original Research Report

HEMOGLOBIN A1c (HbA1c) LEVELS FROM THE EXAMINATION OF DIFFERENT BLOOD PROPORTIONS IN K2EDTA TUBES USING AN ENZYMATIC METHOD

Museyarah , Musholli Himmatun Nabilah , Retno Werdiningsih 

Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia

ABSTRACT

Hemoglobin A1c (HbA1c) examination is the gold standard for diagnosing and monitoring diabetes mellitus patients. In the examination, the pre-analytical phase has the most considerable error rate at 61%. One of the contributing factors to errors in the pre-analytical phase is the use of anticoagulants that does not adhere to established guidelines. Additionally, the incompatibility of the sample volume proportions and the anticoagulants in K2EDTA tubes has been observed in numerous cases. The significance of HbA1c testing, particularly in the prevention of diabetes complications, underscores the need for appropriate procedures to be followed throughout the sampling and pre-analytical phases. This study aimed to determine the effect of differences in the proportion of blood sample volume and anticoagulants in K2EDTA tubes on HbA1c levels. This research was conducted from August to September 2022. The research samples were collected from six healthy subjects at the Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. The blood samples were divided into K2EDTA tubes with varying volumes of 1 mL, 2 mL, 3 mL, and 4 mL. The HbA1c levels were then examined at the Clinical Chemistry Laboratory of Politeknik Kesehatan Kemenkes Surabaya. The data were analyzed using a one-way ANOVA test. The statistical test results ($p > 0.05$) indicated that the proportion of samples containing anticoagulants in the K2EDTA tubes did not have any significant effect on HbA1c levels. In conclusion, it is acceptable to utilize K2EDTA tubes with varying blood sample volumes for measuring HbA1c levels.

Keywords: Diabetes mellitus; hemoglobin A1c (HbA1C); pre-analytical phase

***Correspondence:** Musholli Himmatun Nabilah, Department of Medical Laboratory Technology, Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. Email: mushollihimmatur@gmail.com

Article history

• Submitted 17/11/2022 • Revised 16/5/2023 • Accepted 25/8/2023 • Published 10/9/2023

How to cite: Museyarah, Nabiah MH, Werdiningsih R (2023). Hemoglobin A1c (HbA1c) Levels from the Examination of Different Blood Proportions in K2EDTA Tubes using an Enzymatic Method. *Folia Medica Indonesiana* 59 (3), 262-266, <https://doi.org/10.20473/fmi.v59i3.40652>



Copyright: © 2023 Folia Medica Indonesiana.

This is an open-access article distributed under the terms of the Creative Commons Attribution License as stated in <https://creativecommons.org/licenses/by-nc-sa/4.0/deed.id>.

pISSN:2355-8393, eISSN: 2599-056x

Highlights:

1. Research on the effect of blood volume proportion in the examination of HbA1c levels using K2EDTA anticoagulant has yet to be widely carried out in Indonesia.
2. The analysis conducted utilizing K2EDTA tubes revealed that the varying proportions of blood samples had no effect on HbA1c levels.
3. This article highlights the necessity of evaluating the pre-analytical phase (sample preparation) of a laboratory process to improve the accuracy of the results and minimize false high or low results in the HbA1c test.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by blood sugar levels exceeding normal limits. This disorder can be caused by damage to pancreatic beta cells or a decrease in the functionality of the pancreatic glands. It may lead to either a complete absence or a diminished production of insulin (Infodatin 2020). Globally, this disease is a leading cause of blindness, heart disease, and kidney failure. The International Diabetes Federation (IDF) estimated that at least 463

million people aged 20–79 years suffered from diabetes in 2019 (International Diabetes Federation 2017). According to the results of an Indonesian basic health research project conducted in 2018, the prevalence of diabetes mellitus among individuals aged 15 years was 2%, as determined by medical diagnosis. The results of blood sugar examinations revealed that the prevalence of diabetes mellitus increased from 6.9% in 2013 to 8.5% in 2018. The presented data indicates that only 25% of individuals diagnosed with diabetes are aware of their condition (Infodatin 2020).

Hemoglobin A1c (HbA1c) is the gold standard for monitoring diabetes mellitus patients. The formation of HbA1c bonds occurs slowly and will decompose along with the age of erythrocytes, which is about 3–4 months (Wulandari et al. 2020). The amount of glycosylated hemoglobin depends on the amount of available blood glucose. If blood glucose levels increase for a long time, erythrocytes will be saturated with glucose to produce HbA1c (Utomo et al. 2015). Measurement of HbA1c should be carried out routinely for all diabetic patients, both through the initial examination and as part of ongoing care, to prevent further complications in individuals with diabetes. Three factors influence the interpretation of HbA1c examinations: pre-analytical (patient identification, patient preparation, sampling, sample collection, and sample quality), analytical (sample analysis), and post-analytical (laboratory results) (Gupta et al. 2014, Yaqin 2015).

In a study by Yaqin (2015), it was found that HbA1c examinations in Indonesia had the most considerable error rate of 61% during the pre-analytical phase. The study further showed that the error rates during the analytical phase and the post-analytical phase were 25% and 14%, respectively. One of the pre-analytical factors that affects the results of the examination is the administration of anticoagulants. According to the guidelines from the Clinical and Laboratory Standards Institute (CLSI) in 2004, procedures for processing blood specimens in vacutainer tubes containing blood volumes higher or lower than the recommended levels must be rejected. Too little or too much anticoagulant can affect the results of the study. In addition, the difference in blood volume for all types of vacutainers should not be more than 10% of the recommended volume (Dayalan et al. 2020).

According to a study by Cahyani (2018), the proportion of blood samples filled within the K2EDTA vacutainer tube had an apparent effect on the results of complete blood tests. Incompatible proportions of the sample volume with the specified volume standard established by the CLSI are the most common cases regarding HbA1c examinations. The blood volume used in the K2EDTA tube is often less than the standard volume due to various reasons, such as insufficient blood coming out during the blood sampling process. HbA1c examinations play an important role, especially in preventing diabetes complications. Therefore, it is necessary to adhere to the appropriate procedure, which includes the collection and preparation of samples throughout the pre-analytical phase. It can be crucial to obtain accurate examination results in order to improve the quality of service provided to the public. This study aimed to analyze the effect of blood volume proportions in K2EDTA tubes on HbA1c levels as measured using

the enzymatic method. Medical laboratory professionals are expected to give more consideration to the pre-analytical phase in order to obtain accurate examination results and provide better health services to the general population.

MATERIALS AND METHODS

This was a descriptive-analytical study with a cross-sectional design. This study was conducted in November 2022 at Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia. The inclusion criteria included healthy people aged 20–30 years. The exclusion criteria included people who were pregnant and had a history of diseases such as anemia, diabetes mellitus, hypertension, kidney failure, and coronary heart disease (Yedla et al. 2015). The samples used in this study were whole blood with four different sample volume treatments but the same amount of anticoagulant in the K2EDTA tubes. A total of six healthy subjects provided the samples for this study. Prior to sampling, the participants filled out an informed consent form. Blood samples were collected from the participants in 1 mL, 2 mL, 3 mL, and 4 mL volumes in EDTA tubes. The samples were homogenized before measuring the level of HbA1c with the enzymatic method using the Photometer 5010 (Robert Riele GmbH & Co., Germany). This study received approval from the Health Research Ethics Committee of the Ministry of Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia, with certificate No. EA/1329/KEPK-Poltekkes_Sby/V/2022 on 14/11/2022. The participants in this study were asked to fill out an informed consent form. After the participants gave their consent, blood samples were collected to be used as research samples. The confidentiality of the participants' identities was ensured by assigning a unique code to each research sample. In the event of complications occurring during the sampling process, the researchers bore full responsibility for covering the associated medical expenses.

The method used in collecting samples in this study was consecutive sampling. Federer's formula was employed to estimate the number of sample replications with $t=4$. The minimum number of sample replications was obtained six times (Sujarweni 2013). The results of the examination were presented in tables. IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, New York, United States) was used to analyze the data. The obtained data were tested for normality using the Shapiro-Wilk test and for homogeneity using the Lvene test. If the data were normally distributed, an ANOVA one-way block design would be employed. If there was at least one pair of different treatments, then the post hoc test was performed to determine

which pair was significantly different. In contrast, if the data were not normally distributed, Kruskal-Wallis test was used (Sujarweni 2013).

RESULTS

Table 1 presents the results of the examination of HbA1c levels according to the quantity of sample proportions and anticoagulants used in the K2EDTA tubes. The average HbA1c level in the 1 mL blood volume sample group collected in the K2EDTA tube was 6.2 ± 0.43 . Similarly, the average HbA1c levels were 6.1 ± 0.43 in the 2 mL blood volume sample group, 6.1 ± 0.42 in the 3 mL blood volume sample group, and 6.1 ± 0.52 in the 4 mL blood volume sample group.

Table 1. The results of the examination of HbA1c levels.

| Sample code | HbA1c levels (mg/dL) | | | | History of chronic disease |
|-------------|----------------------|--------------------|--------------------|--------------------|----------------------------|
| | 1 mL of sample | 2 mL of sample | 3 mL of sample | 4 mL of sample | |
| HOO1 | 6.7 | 6.2 | 6.3 | 6.5 | None |
| HOO2 | 5.8 | 5.8 | 5.6 | 5.7 | None |
| HOO3 | 6.3 | 6.1 | 5.9 | 5.8 | None |
| HOO4 | 6.7 | 6.9 | 6.8 | 7.0 | None |
| HOO5 | 6.1 | 5.7 | 6.2 | 5.7 | None |
| HOO6 | 5.7 | 5.9 | 5.8 | 6.1 | None |
| Mean±SD | 6.2 ± 0.4 3 | 6.1 ± 0.4 3 | 6.1 ± 0.4 2 | 6.1 ± 0.5 2 | |

This study involved the collection of blood samples from the participants, following predetermined criteria for inclusion and exclusion. Each sample underwent four treatments, consisting of blood volumes of 1 mL, 2 mL, 3 mL, and 4 mL in K2EDTA tubes. The normality test results, as determined by the Kolmogorov-Smirnov test, produced a value that exceeded the significance level of 0.05 ($p=0.20$). The results suggested that the samples exhibited a normal distribution, therefore prompting the application of a one-way ANOVA statistical test to investigate the potential effect of blood volume proportion on HbA1c levels in a blood examination involving the use of anticoagulant in K2EDTA tubes. The statistical analysis utilizing a one-way ANOVA test revealed a significance value above 0.05 ($p=0.20$). The test result suggested that there was no significant association between the blood volume proportions and the HbA1c levels from the examination results employing anticoagulant in the K2EDTA tubes.

DISCUSSION

This study aimed to determine whether varying blood volume proportions in a blood examination using anticoagulants in different K2EDTA tubes

affect the HbA1c levels in the collected samples. An HbA1c examination can determine the average blood glucose level in the previous 1–3 months, assess diabetes control to prevent complications, and assess the effectiveness of therapy modifications after 2–3 months. HbA1c that is produced will be stored and remain present in red blood cells for around three months, aligning with the typical life span of these cells (Putri & Larasati 2013, Que et al. 2015). The formation of HbA1c depends on the glucose concentration in the bloodstream, so the results of the HbA1c examination can provide the estimation of the average blood glucose level for around three months. This occurs when hemoglobin, a protein in red blood cells that carries oxygen throughout the body, combines with glucose in the blood to become glycated (Sulistio & Mutiara 2015, Ramadhan & Marissa 2015).

There are several factors that may affect the examination of HbA1c levels, such as the presence of icteric specimens. Icteric specimens are characterized by elevated bilirubin levels (>5.0 mg/dL) and a yellowish color in the serum. This is due to the accumulation of bilirubin in the body, which is indicative of impaired liver function. Hemolysis, the rapid destruction of erythrocytes, can lead to both intrinsic and extrinsic abnormalities in red or reddish erythrocytes and serum. Additionally, conditions characterized by a decrease in red blood cells, such as anemia, thalassemia, and long-term blood loss, can result in falsely decreased levels of HbA1c. Anemia is characterized by a decrease in the hemoglobin level in the blood. A reduction in hemoglobin levels is typically accompanied by a decrease in erythrocyte count and hematocrit levels (Sherwani et al. 2016).

The results of this study differed from those of a study conducted by Sartika & Hestiani (2019), who found a correlation between blood volume proportion and the results of routine blood tests using anticoagulants in K2EDTA tubes. The observed phenomenon can be attributed to differences in osmolarity between the blood and anticoagulant substances. When blood volume is not collected in accordance with the established guidelines for the EDTA vacuum tube, it can result in cellular shrinkage (Sarihati et al. 2019). The findings of this study indicated that the proportion of sample volume did not have any significant effect on the HbA1c examination results when anticoagulants in K2EDTA tubes were used. The absence of effect could be because the HbA1c examination measures glycated hemoglobin, glycohemoglobin, or glycosylated hemoglobin (GHb). HbA1c is a compound formed through the reaction between glucose and hemoglobin, which is the component of red blood cells responsible for the transportation of blood and oxygen throughout the body.

Consequently, the morphology of red blood cells does not influence the results of the examination (Rawal et al. 2016). According to the findings of this study, it is possible to utilize a sample volume of less than 3 mL in a K2EDTA tube, which typically has a standard size of 3 mL, for the purpose of conducting a HbA1C examination.

Strength and limitations

Extensive research regarding the effect of blood volume proportion on HbA1c levels using K2EDTA anticoagulants has not been widely conducted in Indonesia. The findings of this study can serve as a theoretical foundation for understanding the effect of varying proportions of blood samples on the measurement of HbA1c levels. This study also sheds light on the pre-analytical phase, specifically the sample preparation, and its potential to enhance the accuracy of examination results by reducing the occurrence of falsely elevated or low HbA1c levels. The limitation of this study was the inclusion of healthy participants, which may have influenced the observed HbA1c levels in normal ranges. A larger sample size would have been advantageous to mitigate potential biases in the study findings.

CONCLUSION

Varying proportions of blood samples have no significant effect on HbA1c levels in an examination using an anti-coagulant in K2EDTA tubes and an enzymatic method. Therefore, the use of a K2EDTA tube in the examination of HbA1c levels with varying blood sample volumes remains acceptable.

Acknowledgment

We would like to thank Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia, for their support and funding that made this research possible.

Conflict of interest

None.

Ethical consideration

The ethical approval for this study was obtained from the Health Research Ethics Committee of the Ministry of Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia, with certificate No. EA/1329/KEPK-Poltekkes_Sby/V/2022 on 14/11/2022.

Funding disclosure

The study received research funding from Politeknik Kesehatan Kemenkes Surabaya, Surabaya, Indonesia.

Author contribution

M conceptualized and designed the study, analyzed and interpreted the data, drafted the research article, and critically revised the article for important intellectual content. MHN provided the final approval of the article, study materials, and statistical expertise, as well as secured funding for the research. RW offered comprehensive support in administrative, technical, and logistical aspects while also gathering and organizing the data.

REFERENCES

- Cahyani RWD (2018). Pengaruh jumlah volume darah dalam tabung vacutainer K2EDTA dan K3EDTA terhadap pemeriksaan darah lengkap (thesis). Available at: <https://repository.poltekkesbdg.info/items/show/3129>.
- Dayalan S, Subbarayan D, Radha RN, et al (2020). Underfilled K2EDTA vacutainer on automated haematological blood cell indices to reject or reconsider? *Journal of Clinical and Diagnostic Research*. doi: 10.7860/JCDR/2020/43292.13578.
- Gupta V, Mittal S, Negi G, et al (2014). Under filled di potassium-ethylene di amine tetra acetic acid vacutainers and its effect on automated blood cell indices in healthy blood donors: Is there a need to re-investigate it as a rejection criterion? *Journal of Applied Hematology* 5, 101. doi: 10.4103/1658-5127.141997.
- Infodatin (2020). Tetap produktif, cegah dan atasi diabetes melitus. Infodatin. Available at: <https://www.kemkes.go.id/downloads/resources/download/pusdatin/infodatin/Infodatin2020DiabetesMelitus.pdf>.
- International Diabetes Federation (2017). IDF diabetes atlas eight edition 2017. IDF. Available at: https://diabetesatlas.org/upload/resources/previews/files/8/IDF_DA_8e-EN-final.pdf.
- Putri AES, Larasati T (2013). Hubungan obesitas dengan kadar HbA1c pasien diabetes melitus tipe 2 di laboratorium patologi klinik rumah sakit umum daerah Abdul Moeloek. *Medical Journal of Lampung University*. Available at: <https://juku.kedokteran.unila.ac.id/index.php/majority/article/view/57/56>.
- Que A, Yasa IWPS, Lestari AAW (2015). Gambaran hasil pemeriksaan kadar HbA1c pada penderita diabetes mellitus di Laboratorium Rumah Sakit Umum Surya Husadha Tahun 2013. *Jurnal Medika Udayana*. Available at: <https://ojs.unud.ac.id/index.php/eum/article/view/>

- 20919.
- Ramadhan N, Marissa N (2015). Karakteristik penderita diabetes mellitus tipe 2 berdasarkan kadar HbA1c di Puskesmas Jayabaru Kota Banda Aceh. *Sel*. doi: [10.22435/sel.v2i2.4637.49-56](https://doi.org/10.22435/sel.v2i2.4637.49-56).
- Rawal G, Yadav S, Kumar R, et al (2016). Glycosylated hemoglobin (HbA1C): A brief overview for clinicians. *Indian Journal of Immunology and Respiratory Medicine* 1, 33–36. Available at: <https://www.ijirm.org/journal-article-file/2224>.
- Sarihati IGAD, Karimah HN, Habibah N (2019). Gambaran kadar HbA1C pada pasien diabetes melitus tipe 2 di RSUD Wangaya. *Meditory : The Journal of Medical Laboratory* 6, 88–98. doi: [10.33992/m.v6i2.442](https://doi.org/10.33992/m.v6i2.442).
- Sartika F, Hestiani N (2019). Kadar HbA1c pada pasien wanita penderita diabetes mellitus tipe 2 di Rsd Dr. Doris Sylvanus Palangka Raya. *Borneo Journal of Medical Laboratory Technology* 2, 97–100. doi: [10.33084/bjmlt.v2i1.1086](https://doi.org/10.33084/bjmlt.v2i1.1086).
- Sherwani SI, Khan HA, Ekhzaimy A, et al (2016). Significance of HbA1c test in diagnosis and prognosis of diabetic patients. *Biomarker Insights* 11, BMI.S38440. doi: [10.4137/BMI.S38440](https://doi.org/10.4137/BMI.S38440).
- Sujarweni VW (2013). *SPSS untuk paramedis*. Gava Media.
- Sulistio G, Mutiara H (2015). Pemeriksaan kadar HbA1c pada pasien diabetes melitus tipe 2 dengan obesitas. *Jurnal Kesehatan dan Agromedicine*. Available at: <https://juke.kedokteran.unila.ac.id/index.php/agro/article/view/1230>.
- Utomo MRS, Wungouw H, Marunduh S (2015). Kadar HbA1C pada pasien diabetes melitus tipe 2 di Puskesmas Bahu Kecamatan Malalayang Kota Manado. *eBiomedik*. doi: <https://doi.org/10.35790/ebm.v3i1.6620>.
- Wulandari IAT, Herawati S, Wande IN (2020). Gambaran kadar HbA1C pada pasien diabetes melitus tipe II di RSUP Sanglah periode Juli-Desember 2017. *J Med Udayana*. Available at: <https://ojs.unud.ac.id/index.php/eum/article/view/58263>.
- Yaqin A (2015). Analisis tahap pemeriksaan pra analitik sebagai upaya peningkatan mutu hasil laboratorium Di RS. Muji Rahayu Surabaya. *J Sains*. Available at: <https://journal.unigres.ac.id/index.php/Sains/article/view/591>.
- Yedla N, Kuchay M, Mithal A (2015). Hemoglobin E disease and glycosylated hemoglobin. *Indian Journal of Endocrinology and Metabolism* 19, 683. doi: [10.4103/2230-8210.163211](https://doi.org/10.4103/2230-8210.163211).

