Journal of Applied Chemistry and Biomedical Laboratory Research
https://doi.org/00.0000/0000-000-000
eISSN xxxx-xxxx

Open Access

The Effect of Mild Hemolysis on Serum Glutamic Oxaloacetic Transaminase Enzyme Activity

Mardiana Putri[1], Fitri Fadhilah[1*], Ani Riyani[2]

*Correspondence:
fitirifadhilahssimkesl@email.com

[1] Department of Medical Laboratory Technology, Sekolah Tinggi Analis Bakti Asih, Bandung, Indonesia
[2] Politeknik Kesehatan Kementerian Kesehatan Bandung, Bandung, Indonesia

Received: May 31, 2025
Accepted: June 27, 2025
Published online: June 30, 2025

ABSTRACT

One of the biggest errors in pre-analytics is hemolysis. Hemolysis is a disruption that occurs in the erythrocyte membrane, resulting in the release of hemoglobin. The wavelength at which the parameters measured spectrophotometrically are determined can overlap with the absorption spectrum of hemoglobin. **Materials and Methods:** the analyte test will be disturbed depending on the level of hemolysis. A study has been conducted on the effect of mild hemolysis (0.05 - 0.1 g / dl hemoglobin) in serum on SGOT enzyme activity. The population in this study were patients who underwent examination at the Mediko Farma Laboratory. **Results:** From the results of measuring the activity of the SGOT enzyme in normal serum and in serum with mild hemolysis, the following results were obtained: for the value of SGOT activity in normal serum, the lowest value was 18 IU/L and the highest was 36 IU/L, and the average value was 22.68 IU/L. For the activity of SGOT activity in serum with mild hemolysis, the lowest value was 33 IU/L and the highest was 52 IU/L, and the average value was 39.63 IU/L. **Conclusion:** The percentage of average increase in SGOT activity in serum with mild hemolysis levels was 77.52%. The Wilcoxon test to determine the effect of hemolysis showed that Asymp. Sig (2-tailed) was 0.001, which means that there is an effect of mild hemolysis levels in serum on SGOT enzyme activity.

Keywords: Enzyme; Hemolysis; SGOT

Citation:
Putri M, Fadhilah F, & Riyani A. The effect of mild hemolysis on serum glutamic oxaloacetic transaminase enzyme activity. J Appl Chem Biomed Lab Res (JACBioLab). 2025;1(1):6-9. https://doi

INTRODUCTION

Hemolysis, the rupture of red blood cells, significantly impacts serum SGOT (Serum Glutamate Oxaloacetate Transaminase) levels due to the release of intracellular enzymes into the bloodstream. Research demonstrates that hemolysis introduces pre-analytical inaccuracies, leading to falsely elevated SGOT readings [1,2].

One of the biggest mistakes in pre-analytics is hemolysis. Hemolysis is a disorder that occurs in the erythrocyte membrane, resulting in the release of hemoglobin. Hemolysis can cause effects on many laboratory tests such as potassium, sodium, calcium, bilirubin, magnesium, total protein, LDH, AST, ALT, phosphorus, ALP, acid phosphatase, GGT, folate, iron [1-3].

Laboratory test results are influenced by the pre-analytical, analytical and post-analytical stages. The largest contribution of errors in clinical laboratories is at the pre-analytical stage of 62%, while the analytical and pre-analytical stages are 15% and 23%. The pre-analytical stage contributes the largest error because it is directly related to the patient so it is difficult to control. Some things that can cause pre-analytical errors include hemolysis

(53%), insufficient specimen volume (7.5%), illegible handwriting (7.2%), wrong specimen, clots in the specimen, errors in the vacutainer or anticoagulant, inappropriate ratio of specimen volume to coagulant and specimens taken from the infusion line [3,4].

The possibility of hemolysis must be minimized when taking samples. Hemolysis must be avoided because it can affect the test results. Hemolysis is a condition in which the red blood cell membrane is ruptured and hemoglobin is released. Hemolysis occurs when the serum hemoglobin concentration exceeds 20 mg / dl [3,5].

Mild hemolysis classification in plasma is pink plasma, and plasma-free hemoglobin ranges from 0.5 g/L – 1.0 g/L, moderate hemolysis in plasma is red plasma, and plasma-free hemoglobin ranges from 4.0 g/L – 8.0 g/L, severe hemolysis in plasma is dark red plasma, and plasma-free hemoglobin ranges from 16.0 g/L – 32.0 g/L [6,7].

Hemolysis artificially elevates serum SGOT (aspartate aminotransferase) levels through the release of intracellular enzymes from ruptured red blood cells. This pre-analytical interference occurs because erythrocytes contain SGOT, albeit at lower concentrations than liver or cardiac cells [5,8,9].

MATERIALS AND METHODS

Hemolysate Preparation

The method of making hemolysate is the sample obtained is put into EDTA as much as 1.5 ml. The tube is centrifuged for 15 minutes at a speed of 1500 rpm. Then the solid phase is taken. The solid phase is washed with 0.9% NaCl so that concentrated erythrocytes are obtained. Erythrocytes are lysed with distilled water. The use of distilled water is as a hypotonic fluid in which erythrocyte cells will experience swelling because distilled water seeps into the cells through the cell membrane. Finally the cells will lyse. This erythrocyte concentrate is used as a hemolysate with six different levels of hemolysate. Where hemolysate level 1 (H1) is 5 µl of erythrocytes plus 1000 µl of distilled water, hemolysate level 2 (H2) is 10 µl of erythrocytes plus 1000 µl of distilled water, hemolysate level 3 (H3) is 20 µl of erythrocytes plus 1000 µl of distilled water, hemolysate level 4 (H4) is 60 µl of erythrocytes plus 1000 µl of distilled water, hemolysate level 5 (H5) is 100 µl of erythrocytes plus 1000 µl of distilled water, and hemolysate level 6 (H6) is 150 µl of erythrocytes plus 1000 µl of distilled water. The six levels of hemolysate were examined for their hemolysis index using the cyanmethemoglobin method [6].

SGOT Enzyme Examination

SGOT examination is carried out using the spectrophotometric or photometric method using a spectrophotometer or automatic chemical tool. The examination material used is serum or heparin plasma. The most commonly used SGOT examination method today is the enzymatic reaction kinetic method using the UV optimization test according to WHO/IFCC standards. This method consists of 2 types, namely the IFCC method with the addition of pyridoxal phosphate reagent or commonly called the IFCC method with PP or substrate start or the IFCC method without the addition of pyridoxal phosphate reagent or commonly called sample start or IFCC without PP.

RESULTS AND DISCUSSION

This study is an experimental study using normal serum samples and serum that has been processed into hemolyzed serum. The applied research design is static-group comparison, namely a group that has undergone a certain treatment compared to a group that has not. The observed differences between the two groups are assumed to be a result of the treatment. In this design, there are two groups that are determined as research subjects. One group receives treatment and one group does not receive treatment.

Hemolysate Production Results

Concentrated hemolysate concentration 8 g/dL. Dilute first 10x so that the hemolysate volume is not too small (adjusted to the existing micropipette) so that 0.8 g/dL hemolysate is obtained. 60 µl of hemolysate is taken, added to 440 µl of serum replaced with 60 µL of hemolysate as the final volume remains 500 µL. Serum with hemolysate 0.096 g/dL is obtained. Serum with hemolysate is used as a sample and the results of measuring SGOT activity in this sample are compared to serum without hemolysate.

SGOT measurement

Normal SGOT activity: 13 – 31 IU/L (Biolabo SGOT reagent insert kit), then the data obtained that for the SGOT activity value in normal serum, the lowest value is 18 IU/L and the highest is 36 IU/L, and the average value is 22.68

IU/L. For the activity of SGOT activity in serum with mild hemolysis, the lowest value is 33 IU/L and the highest is 52 IU/L, and the average value is 39.63 IU/L. The average percentage increase in SGOT activity in serum with mild hemolysis was 77.52%.

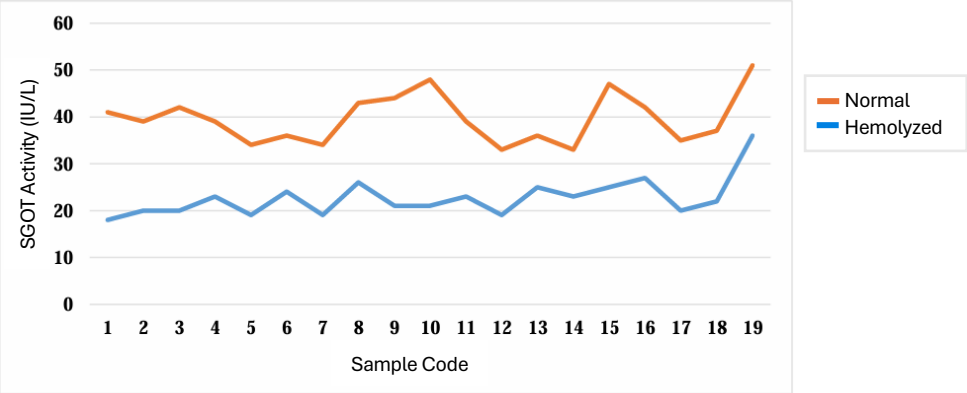

Figure 1. Comparison of SGOT Activity in Normal and Hemolyzed Serum

CONCLUSIONS

Clinically, this difference can be considered significant because there is a supporting theory that SGOT enzyme activity in hemolyzed serum can cause a false increase caused by the release of SGOT enzyme found in erythrocytes, so that SGOT enzyme activity in hemolyzed serum is theoretically higher than normal serum [10,11].

But there are also those who argue that in vitro hemolysis is a special concern when blood is collected for diagnostic testing because it has the potential to cause incorrect measurements of some analytes, there is interference in the measurement at a predetermined wavelength. As in this SGOT measurement which is carried out at a wavelength in the UV region, namely 340 nm. When the solution becomes colored, interference will occur. The wavelength at which the parameters measured spectrophotometrically are determined can overlap with the absorption spectrum of hemoglobin. Thus, analyte testing will experience interference depending on the level of hemolysis. Both oxyhemoglobin and deoxyhemoglobin have maximum absorption at 415 nm with a detection range between 320 nm and 450 nm, and between 540 nm and 589 nm. Tests whose detection wavelengths are between the above intervals will be affected. In the SGOT examination insert kit from Biolabo, the IFCC method states that positive interference (an increase in enzyme activity measurement results, in the form of a false positive) will be detected if the hemoglobin level in the serum is 114 µmol/L [3,5,6,11].

Traditionally, specimens were detected for hemolysis by visual inspection, often at random, a practice that is currently discouraged due to unreliable and variable results. Today, almost all biochemical analysis platforms are equipped with hardware and software capable of detecting analytical interferences. One such indicator is the hemolysis index, a quantitative value associated with the amount of free hemoglobin in plasma or serum. Because of its ability to detect mild hemolysis and the potential for consistent detection methods across laboratories, the hemolysis index is now considered the best practice for hemolysis detection. Although it is known that the effect of hemolysis interferences on test results depends on the analyte being tested, there is little information on the consistency of hemolysis detection and reporting methods among individual laboratories [3,6,7].

Author Contributions: Con-ceptualization, FF; methodology, MP; software, MP.; validation, FF.; formal analysis, MP; investigation, MP; resources, FF.; data curation, MP; writing—original draft preparation, FF, MP .; writing—review and editing, FF; visualization, FF,MP; supervision, FF; project administration, FF; All authors have read and agreed to the published version of the manuscript.

Funding: Sekolah Tinggi Analis Bakti Asih internal funding.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Acknowledgments: The authors have reviewed and edited the output and take full responsibility for the content of this publication.

Conflicts of Interest: The authors declare no conflicts of interest.

REFERENCES

1. Al-Kaleel A, Al-Gialani L. Sourcebook update: RBC hemolysis studies using a simple modified blood film technique. Adv Physiol Educ. 2024;48(2):427–9.
2. Goodhead LK, MacMillan FM. Corrigendum to: Measuring osmosis and hemolysis of red blood cells [Adv Physiol Educ, 41 (2017) (298-305)] doi: 10.1152/advan.00083.2016. Adv Physiol Educ. 2018;42(3):527–527.
3. Dila Wanti H, Fadhilah F, Taufiqurrohman O, D-III Analis Kesehatan P, Tinggi Analis Bakti Asih S, Barat J. Pengaruh hemolisis dalam serum terhadap aktivitas enzim aspartat aminotransferase dengan metode kinetik-IFCC. J Indones Med Lab Sci. 2020;1(1):48–56.
4. Goodhead LK, MacMillan FM. Measuring osmosis and hemolysis of red blood cells. Adv Physiol Educ. 2017;41(2):298–305.
5. Fadhilah F, Rahmawati I, Anggraeni N, Riyadi SA. Temperature Effects on Plasma Li-heparin and Transaminase Activity in Children Blood with Tetralogy of Fallot. In: Journal of Physics: Conference Series. 2021.
6. Ariyani L, Siagian LR, Indriaty Yusran D, Kesehatan A, Kemenkes Kaltim P, Patologi Klinik RSUD Abdul Wahab Sjahrani L. Effect of hemolysis index on improvement of glutamate oxaloacetat transaminase (SGOT) serum. Husada Mahakam. 2019;9(2):42–50.
7. Hartono K. Pengaruh Hemolisis Terhadap Kadar Serum Glutamate Pyruvate Transaminase (SGPT) Sebagai Salah Satu Parameter Fungsi Hati. 2018.
8. Fibriani A, Wisaksana R, Alisjahbana B, Indrati A, Schutten M, van Crevel R, et al. Hepatitis B virus prevalence, risk factors and genotype distribution in HIV infected patients from West Java, Indonesia. J Clin Virol. 2014;59(4):235–41.
9. Fatima K, Moeed A, Waqar E, Atif AR, Kamran A, Rizvi H, et al. Efficacy of statins in treatment and development of non-alcoholic fatty liver disease and steatohepatitis: A systematic review and meta-analysis. Clin Res Hepatol Gastroenterol. 2022;46(4).
10. Handayani I, Rengga Indrati A, Alam A, Ilmu Kesehatan Anak D, Patologi Klinik RSUP Hasan Sadikin DD. Profil Manifestasi Klinis dan Laboratoris Pasien Dengue Bayi yang Menjalani Rawat Inap di RSUP. DR. Hasan Sadikin Bandung. Sari Pediatr. 2018;19(3):119–26.
11. Tamber SS, Bansal P, Sharma S, Singh RB, Sharma R. Biomarkers of liver diseases. Mol Biol Rep. 2023;50(9):7815–23.