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Plasma Absorbance As The Baseline: A Simple Method For Correcting The Results Of Blood Glucose Level On Hemolyzed Specimens

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Abstract. Hemolysis on laboratory specimens can be caused by many factors, in vivo, and in vitro. Increased levels of free hemoglobin in the serum specimen can influence the measurement results of the various test. Hemolysis is often difficult to detect properly. Even for serum specimens that appear to be hemolyze, the laboratory does not have a good protocol to avoid errors. This study aims to develop a simple approach that can be carried out in correcting the measured results of laboratory assays. A correction by calculating the plasma absorbance was applied to blood glucose results from hemolyzed specimens. The correction results were then compared with the levels from the same specimens that not hemolyzed. This study was carried out using the experimental method on blood specimens collected from 15 volunteers. Specimens were treated mechanically to produce some specific hemolysates by forced suppressing the blood through a syringe with 23G needle, in 0 to 6 times. In addition to measuring glucose levels, absorbance measurements were also carried out for each specimen diluted in a buffer solution to calculate the increase in light absorbance as the plasma absorbance. All measured data were analyzed using the Repeated Measure ANOVA. In this study, the increase in average levels was occurred according to the frequency of treatment given. The differences in the increasing results of each treatment before being corrected were statistically significant ($p < 0.01$). However, after the measurement baseline was corrected by plasma absorbance, then the difference in all results turned out to be insignificant. ($p > 0.05$). It can be concluded that the increase in light absorption due to free hemoglobin concentration is the cause of increased results from each hemolysate. However, the measurement of plasma absorbance has been proven as an appropriate procedure for correcting the result of glucose level from hemolyzed specimens before being reported.

Keywords: hemolyzed specimens, correction, absorbance, glucose

I. INTRODUCTION

Proper laboratory diagnosis is very important to get the best results from the examination. Much of the research that has been done in recent years aims to reduce errors in laboratory diagnoses. Diagnostic errors occur in laboratory testing, especially the pre and post-analysis phases, which occur more frequently than errors in the analytic phase. This shows that laboratories need to refocus their efforts on reducing errors in the whole testing process, not just the analytical phase. The research emphasized the importance of focusing on the use of appropriate tests and the interpretation of accurate results to reduce the risk of diagnostic errors related to the laboratory as an effort to improve patient care [1,2].

Good specimen collection and processing techniques have been emphasized on laboratory staff to reduce errors in the pre-analytic stage. At present in accurate collection of specimens has been significantly reduced. However, the most recent data about errors in the pre- analytic phase, especially in the initial procedures that are partly not done in clinical laboratories, such as in the emergency department, or not under the control of laboratory staff. The data also shows an error in requesting the appropriate test parameters and the inadequacy of the specimen sent to the laboratory, such as hemolysis, due to inadequate specimen collection techniques [2,3]. In the post-analytic phase, the most common mistakes are excessive turn around times, errors in the result data entry and skipping corrections from incorrect findings.

Incompatible results are often found in laboratory diagnosis at the verification stage. Lack of knowledge and attention from laboratory staff in carrying out confirmation of diagnostic results is still a major problem in the delivery of services [4].

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Hemolysis, as mentioned earlier is often encountered due to improper specimen collection techniques. This condition requires the laboratory to make good specimen selection, even refusing if it does not meet the required criteria. The presence of hemolysate significantly affected LDH and AST at almost undetectable hemolysis by visual inspection (i.e., free hemoglobin in plasma <0.5 g/L). But the condition of hemolysis can also occur in patients with certain diseases such as hemolytic anemia, sickle cells, thalassemia, infectious diseases, and others. This sometimes causes the laboratory to continue to have an examination even though the hemolyzed specimens obtained can cause interference with the determination of the levels of some of the examination parameters [4,5].

Based on research by Lippi Giuseppe, et al. (University study in Verona, Italy, 2006) which shows that the condition of hemolysis causes significant biases in many clinical laboratory test results. However, the differences in test results from hemolyzed specimens are still within acceptable limits in several test parameters, such as in measuring the activity of enzymes that are not contained in red blood cells [6]. The mechanism of these includes the release of the contents of intracellular to plasma/serum, increased absorption of light by hemoglobin, as well as the effect of the chemical on the contents in the blood [7].

In metabolite level determinations, especially Trinder reaction-based assays such as glucose, hemolyzed specimens generally show a significant increase caused by the elevate in the rate of light absorption due to the high free hemoglobin content. It seems to be able to remove the effect by taking a separate measurement of the reading of the tested solution to the absorbance value of hemolysate. It is expected to prove that recording the results after considering the plasma absorbance rate of the hemolyzed specimen as a baseline for calculation is an easier, faster, and more precise action to improve examination results.

II. RESEARCH METHODHOLOGY

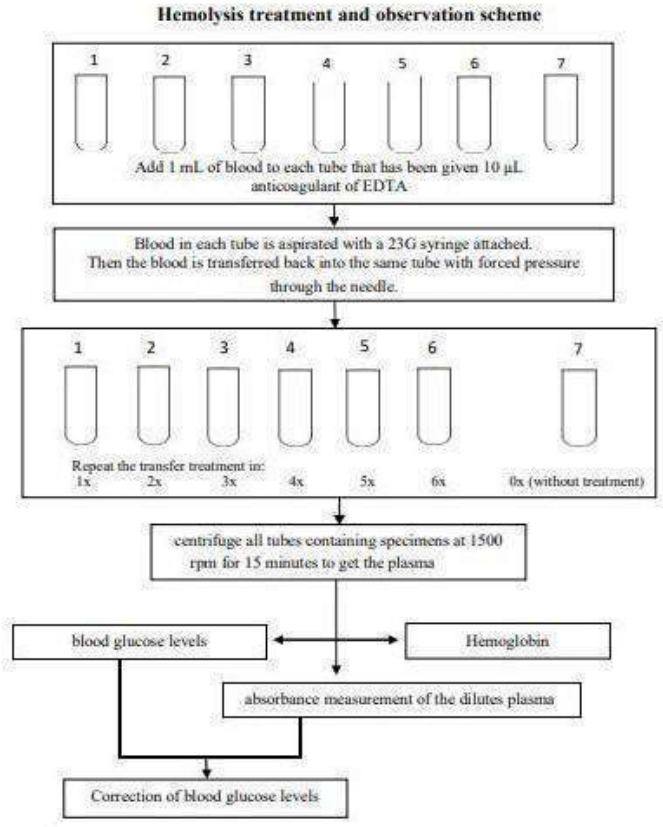
The study was conducted at the Chemical Clinical Laboratory of the Medical Laboratory Technology Department, Health Polytechnic of Ministry of Health in Jambi on August 2019. The examination was carried out on blood samples obtained from 15 healthy participants who were students of the academy. All participants volunteered to be involved in this study and each was asked to sign a consent form before conducting blood collection.

The blood specimens obtained from each participant were divided into 7 tubes, each containing 1 mL. The tubes had previously been added by EDTA anticoagulant in the amount of 1 mg each. The first tube was used as a control plasma, while each blood in the other six tubes was treated with hemolysis by a forced pressure transfer through a 23G syringe needle. The

transfers were performed repeatedly at 1 to 6 times in succession. Plasma separation was carried out by centrifugation at 1500 rpm for 15 minutes. Determination of blood glucose levels is carried out using Glucose Oxidase - Aminoantypirin Peroxidase (GOD-PAP) method. Absorbance measurements and reading of the results of the measurement carried out photometrically using the Erma AE-600N photometer.

Blood specimens are treated for hemolysis and observed base on the scheme below.

Hemolysis treatment and observation scheme



Determination of blood glucose levels

Insert the working reagent into a tube of 1 mL and add 10 µL of plasma fluid from each tube to be observed. Glucose level was measured using AE-600N spectrophotometer at a wavelength of 505 nm by comparing it to the absorbance of glucose standard solution and calculating the absorbance blank reagent. The results of the grade and absorbance values that appear on the monitor screen on the device are well recorded.

Determination of Hemoglobin

Add as much as 1 mL of drabkins reagent to each prepared tube. Add the serum that has been treated as much as 100 µl, then measure the Hb level with the AE-600N spectrophotometer using the factors present in the tool (determined by a previous calibration curve) at a wavelength of 505 nm. The results obtained are then divided by 10 as a multiple factor of the volume of the specimen that has been applied to get the true value.

Measurement of plasma absorbance

Add as much as 1 mL of 0.9% NaCl solution into the tube, then add 50 µL of plasma as they are measured. Absorbance was measured using AE-600N spectrophotometer. To get the true value, the absorbance displayed on the device monitor is properly recorded and divided by 5 as a factor of the multiple of the specimen volume that has beenapplied.

Statistic analysis

Data from spectrophotometric measurements were analyzed and presented descriptively in tables and graphs based on the average value of each treatment. The hypothesis was evaluated using a one-way ANOVA Repeated Measure with a significance level of 5% and continued with a comparative test to see the significance of the differences between treatments (DMRT test). Statistical analysis was performed using Minitab 17 software.

III. RESULTS AND DISCUSSIONS

From the research data that has been obtained, hemolyzed specimens that are not corrected for absorbance in blood samples have an increased impact on blood glucose examination results. However, after examining the plasma absorbance and correcting blood glucose levels using a hemolysis sample, it turns out that there is no significant difference in the results of blood glucose as shown in Table 1. The statistical test used for the design of this study is the one-way test Anova for repeated measure with one type of treatment.

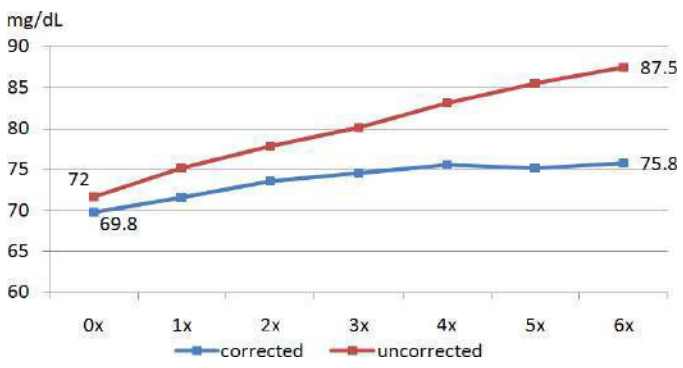
Anova one-way repeated measures test carried out on the results of examination of glucose levels that were not corrected by plasma absorbance showed a very significant difference ($p < 0.01$). This is demonstrated (see Table 1) by a very sharp increase in the average value of specimens with no hemolysis treatment, from 71.7 mg / dL to 87.5 mg / dL of the same specimen after 6 times of repeated treatment. This significant difference has occurred in the first hemolysis treatment (1-time treatment) which is shown from the results of the DMRT test analysis. This also proves that if no absorbance correction is made from the hemolysis specimen, there is a significant increase in levels.

Table 1. Blood glucose levels of hemolyzed specimens with different treatment variations before and after corrected by plasma absorbance**.**

Hemolysis treatment	**before corrected (mg/dL)**			**after corrected (mg/dL)**		
		Range	$\bar{x}$		**Range**	$\bar{x}$
0 (non-hemolysis)	56.3	- 93.4	71.7	55,3	- 91,1	69,8
1 time	60.8	- 94.5	75.2	58,8	- 89,3	71,6
2 times	62.3	- 95.7	77.8	59,1	- 89,5	72,0
3 times	69.3	- 97.1	80.2	65,9	- 89,3	74,5
4 times	77.2	- 99.7	83.1	70,7	- 88,8	75,6
5 times	79.7	- 98.3	85.5	66,6	- 83,8	75,2
6 times	82.0	- 101.4	87.5	68,6	- 86,5	75,8
x	69,7	- 97.2	80.1	63,5	- 88.3	**73,5**

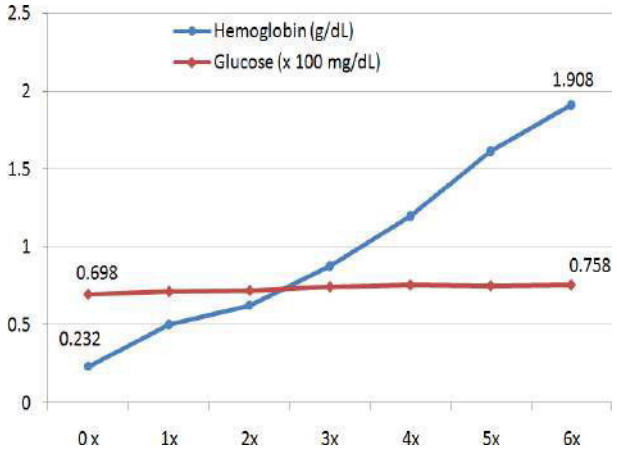
However, after the procedure of plasma absorbance correction was performed on the results as a baseline of hemolysed specimens measurement, the p-value was 0.132, which means that there were no significant differences anymore. The average value of the specimen (non- hemolysis) which showed a result of 69.8 mg / dL only increased slightly to 75.8 mg / dL after the same specimen became hemolysis with repeated treatment 6 times. So it can be interpreted that the hemolysate has no effect on blood glucose levels if the baseline was corrected with the absorbance of theplasma.

Figure 1. The difference between the average corrected and uncorrected blood glucose levels



The higher the repetition of the treatment given, the higher the average value of glucose levels obtained. The magnitude of the difference in increase in the levels of each treatment can be observed in the figures in table 1 above. The difference between specimens that did not undergo hemolysis with specimens that were treated with the highest hemolysis (6 times) reached an average of 15.8 mg / dL. Even in previous studies found an average increase in blood glucose levels with hemolysis treatment as much as 0-20x increased to 60,342 mg / dL. In this study after baselie-correction of plasma absorbance of hemolyzed specimens, an increase of only 6.0 mg / dL of the specimen was treated 6 times.

Figure 2. Comparative graph of elevated levels of Hemoglobin and Blood Glucose Levels after corrected for plasma absorbance



The more treatment is given, the more blood is experienzcing hemolysis. In this study the average value of hemoglobin in plasma due to the highest hemolysis treatment was 1.91 g / dL. This condition is indicated by an increase of free hemoglobin in plasma. When compared with changes in the results of blood glucose levels, it is clear that the hemolysate does not interfere with the results obtained from the corrected glucose measurement. Thus, laboratory tests of hemolyzed specimens can be assured of reliable results if appropriate corrective procedures are performed.

The manual calculation that is carried out with the initial formula of end-point measurement, the correction is applied to the same calculation formula by subtracting the absorbance rate of the measured specimen (absorbance of the test) with the absorbance rate of diluted hemolysis plasma (in NaCl solution). In a semi-automated or fully-automated system: the result can be directly corrected by subtracting the value of the initial result that shows at the monitor device with the value of the measured result of hemolysis plasma after being divided by 5 (multiplication factor number).

IV. CONCLUSIONS

This study concluded that the implementation of the baseline-correction with the plasma absorbance from hemolyzed specimens can be applied as a standard procedure in clinical laboratory assays. During this time, many laboratories may not examine the specimen when they found this condition, but in according to this discovery of the facts which obtained in this study, it is necessary to consider other actions that must be taken. It might be harmful to the patient due to re-collection in case of rejecting the specimen, on the contrary, if the examination is continued to be carried out, then the results will be unreliable if it was performed without considering the correction and may cause a diagnostic error by theclinician.

Maybe a similar circumstance will also be found if applied to other laboratory tests, especially those using a method similar to this glucose determination method, such as testing of cholesterol, triglyceride and uric acid levels which also use peroxidase enzymes with the same Trinder reaction which product quinone dye compounds.

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